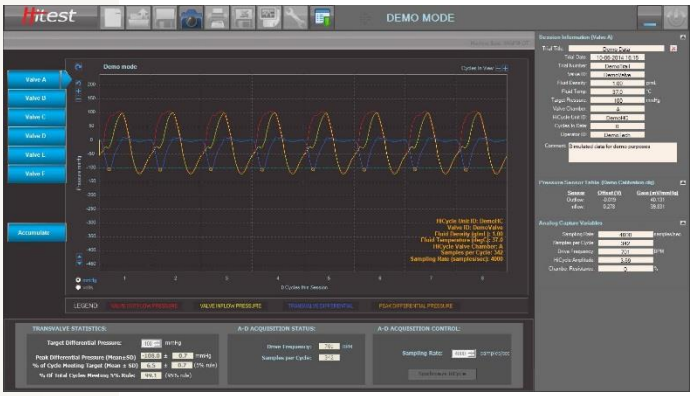
HiCycle System
AR Series
User Manual
HiTest Version 5.0



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ViVitro Labs HiCycle System
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1. About ViVitro Labs

ViVitro Labs Inc. offers industry leading cardiovascular test equipment and related laboratory testing services. Hundreds of organizations in over 39 countries for 30+ years have trusted ViVitro's expertise, accuracy, and quality for their cardiovascular device testing.

As the developer of the world's first Pulse Duplicator, the ViVitro name has been synonymous with cardiovascular device testing equipment. ViVitro hardware and software products have been used by leading R&D facilities and academic labs worldwide, and its equipment and testing methods have been cited in hundreds of peer reviewed publications (http://ViVitrolabs.com/company/cited-publications/). The ViVitro Labs' Pulse Duplicator System (in particular the ViVitro Labs SuperPump) has become a worldwide standard to simulate physiological heart conditions in academic research applications

ViVitro Labs Inc., holds ISO/IEC 17025 accreditation for laboratory testing services endorsed by A2LA and based on ISO 5840. This scope of accreditation includes the physical and mechanical testing of heart valve substitutes including durability, hydrodynamic, and flow visualization testing.

ViVitro Labs is widely recognized as the authority on cardiovascular device testing. ViVitro Laboratory Services are engaged worldwide for hydrodynamic, durability, and flow visualization testing of heart valves and other cardiac devices. ViVitro has been the trusted name in regulatory approvals for over 30 years and is renowned for its proven success from product development testing through to full regulatory submission.

ViVitro Labs is a member of the ISO 5840 standards committee and is actively engaged in developing regulatory requirements. Leveraging this intimate knowledge of the standard, ViVitro Labs ensures that test protocols will meet the ever changing regulatory requirements. ViVitro's Laboratory Testing Services offers an ISO/IEC 17025 certified lab using ViVitro equipment, to conduct 3rd party independent testing. ViVitro's accredited testing lab is governed by a mature Quality Management System (QMS) certified to meet the ISO 5840. This gives assurance to stakeholders and regulatory bodies that results are obtained by qualified personnel using traceable calibrated equipment and up to-date test methods, all supervised by a quality assurance department.

1.1 About This Manual

This document describes the HiCycle AR System's procedures for:

- Assembly of the system's hardware
- System setup
- Operator procedures for the system's daily use
- HiTest software operating instructions

For further information on the optional Data Acquisition System, please see the Data Acquisition System user manual.

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1.2 About HiCycle AR Series System Hardware

The HiCycle System assesses the durability of prosthetic heart valves and other cardiovascular products and biomaterial specimens. This assessment can be used to determine the valve's continued function over a reasonable lifetime. The HiCycle System provides accelerated life cycle testing of up to 6 heart valves or biomaterial specimens at a time. This is a laboratory test instrument not designed for use on humans or animals.

The HiCycle System uses an electro-dynamic shaker to drive 6 pistons within fluid-filled, temperature-controlled chambers. The pulsatile flow exercises mounted heart valves or specimens at an accelerated rate of up to 1,650 cycles/minute. Clear viewports on each chamber allow imaging of the valves during testing. Pressure transducers allow monitoring of the dynamic pressures up and down stream of the valves with the use of HiTest Software.

The ViVitro Labs Inc. HiCycle AR Series System's features include:

- Simultaneous testing of 2, 3, 4 or 6 samples
- Adjustable cycle rate from 500 to 1,650 cycles/minute
- Independent, but not simultaneous, monitoring of each sample chamber for inflow, outflow, and trans-valve pressures
- Individual flow control for each sample chamber
- Sample observation and illumination using a stroboscope through non-distorting viewports
- Sample positioning/locating without major disassembly
- Flexible sized sample mounting using adapter rings up to 50 mm in height and 35 mm in diameter
- Controlled temperature from ambient to 40ºC ±0.5°C
- Grand Total, Total, and Batch Count functions
- Custom designed cart to allow the equipment to be free standing and mobile
- Fluid contact areas made of corrosion resistant materials
- Pressure Head to the sample chambers ranging from 0 to 65 mmHg (8.7kPa)

The HiCycle System is comprised of:

- A test Module (Drive Unit) which creates pulsatile flow through up to 6 test samples
- An electronic Controller which drives the Module and can provide the link to the HiTest monitoring software

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HiCycle AR Series Hardware comprised of the Module (left) and Controller (right)

Optional components:

- HiCycle Cart - Houses the Controller and Module at a convenient working height
- Stroboscope – Allows user to view valve operation while testing at high cycle rates
- Digital Manometer – Used for system pressure calibration

HiCycle Hardware installed on the optional Cart

Stroboscope

Digital Manometer

- HiCycle Data Acquisition System – Comprised of the AmPack, I/O Module, and a Laptop with HiTest Software, the HiCycle Data Acquisition System complements the HiCycle System by monitoring and recording pressure data.

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HiCycle Data Acquisition System comprised of the Laptop (left), AmPack (top right) and I/O Module (bottom right)

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2. Cautions

- Caution should be used when lifting the Module. Injury can occur from improper lifting of heavy loads.
- Do not lift or move the Module by handling the top plate. This can result in misalignment of the system.

- The HiCycle Module must be fastened to the mounting location (optional cart or secure bench) using the threaded anchor points on the Module base plate.
- The HiCycle should be returned to ViVitro Labs in the case of the unit being dropped or other physical damage.
- Do not remove the cover from the electronic Controller. There are no user serviceable parts inside.
- Do not use solvents on any components.
- Always use the power cord supplied with the unit to ensure proper grounding.
- Ensure the rear of the Controller is unobstructed to provide adequate ventilation.
- The amplitude must be reduced to minimum before starting initial operation to prevent damage to the Module or test samples.
- It is not recommended to run the system at greater than 1080 bpm (18 Hz) due to loss of accuracy of pressure readings.
- Changes or modifications to this instrument not expressly approved by ViVitro Labs will void the product warranty and could cause safety issues.

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3. System Assembly

3.1 List of Components

The following components should be identified on unpacking:

Box 1

- HiCycle Module
- Scan Valve handle (affixed to top of Module)
- HiCycle Accessories Kit
- Pressure Head Assembly (mounting pole, syringe, catheter tubing)

Box 2

- HiCycle Controller unit
- Control cable
- Power cord
- HiCycle Cart (optional)
- Two (2) large thumb screws (optional)

Box 3 - HiCycle Data Acquisition System (optional)

- ViVitro Laptop with HiTest Software
- AmPack Pressure Measuring System
- I/O Module
- Connecting cables
- Stroboscope (optional)
- Manometer (optional)

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3.2 Unpacking

Inspect the shipping container for external damage. Retain the shipping carton and packing materials for possible re-shipment. Inspect the system components for damage. If there is evidence of damage contact the carrier, initiate a damage claim, and inform ViVitro Labs.

CAUTION: INJURY CAN OCCUR FROM IMPROPER LIFTING OF HEAVY LOADS. DO NOT MOVE MODULE BY HANDLING VALVE TEST CHAMBERS.

The Module weight is 25 kg (55 lb). It should be unpacked carefully as follows:

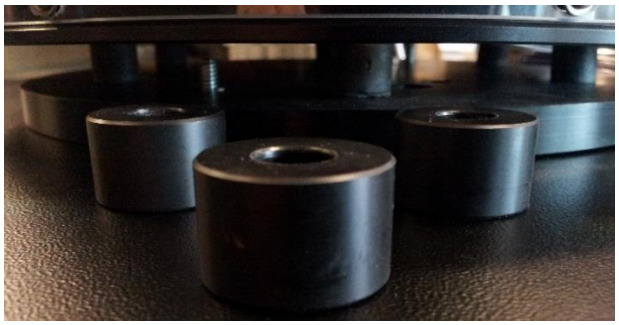
1. Lift the inner shipping crate out of the box using the handle on the top and place on firm surface.
2. Remove 4x phillips head screws from the two sides with the “Unscrew here first” labels.

3. Lift off the top of the crate box off of the base using the handle on top of the crate.

4. Remove shipping bolts under the crate base releasing the Module.

5. Lift the Module to the mounting location (optional cart or secure bench). DO NOT LIFT BY THE MODULE CHAMBERS. Lift the HiCycle by the bottom plate of the Module.

6. Remove three black shipping spacers beside the rubber Module base plate isolation mounts and store with the shipping crate for future shipping.



7. Remove packing materials from the Module.

8. Unpack Controller, power cable, and connection cable.

Inspect the Module and Controller for external signs of damage in shipment. The alignment of many internal components of the HiCycle Module is critical. If there are signs of damage, initiate a claim with the carrier and inform ViVitro Labs.

3.3 Hardware Assembly

This section provides the user with the procedures for the hardware assembly of the HiCycle System.

NOTE: The installation site for the HiCycle must have a properly grounded 90-240 VAC / 50-60Hz power supply and a sturdy table or bench if the optional Cart is not being used.

1. Place the Controller on the Cart shelf (optional) with the device's controls in the elevated position. Ensure there is adequate ventilation and access to the power switch at the rear of the device.

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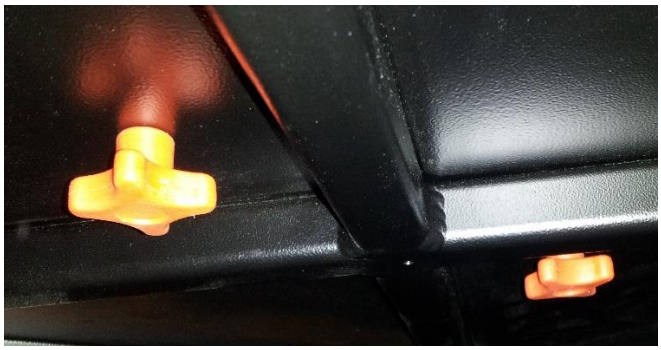
2. Place the Module on the top of the Cart (optional), carefully positioning the unit over the dowel pin and bolt holes. Orient the connector on the Module toward the front of the Cart.

3. Use the two large thumb screws to secure the device to the Cart. Do not over tighten.

   NOTE: If mounting the HiCycle on a bench THE HICYCLE MUST BE FASTENED TO THE BENCH USING THE THREADED ANCHOR POINTS ON THE BASE PLATE.



4. Feed the pressure transducer cables through Scan Valve handle and strain relief hose.

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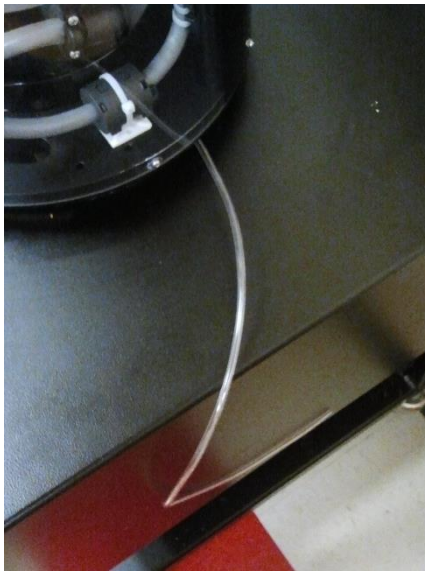
5. Locate the Scan Valve handle on two red thumb screws on the top of the module. Offset the two white spacers to retain the Scan Valve handle as shown below, then secure using the two red thumb screws.

6. Unravel the overflow drain tube at the base of the Module and position it to drain spilled fluid from the Scan Valve away from the Module and Controller.



7. Connect the 25 pin cable to the Controller and the Module. Secure by tightening the two screws on each connector with a small flat blade screwdriver. Do not over tighten.

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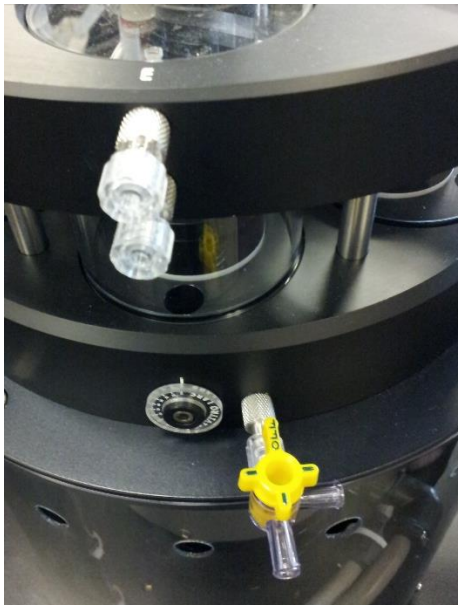
8. Install six (6) stopcocks from the Accessories Kit on the lower luer fittings of the Module.



9. Thread the two Pressure Head Assembly rods together.

10. Add a stopcock from the Accessories Kit to the Pressure Head Assembly syringe. Cap the open port of the stopcock with a cap from the Accessories Kit.

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11. Insert the Pressure Head Assembly in the opening in the rear left of the Cart (optional). Secure using the small thumb nut.

12. Connect the catheter line to the bottom stopcock of one of the test chambers.

13. Connect the Controller to the power supply using the cable provided.

If connecting a HiCycle Data Acquisition System (optional), connect as described below. An alternate configuration, which mounts the transducers to the outside of the Module, is shown in section 13.

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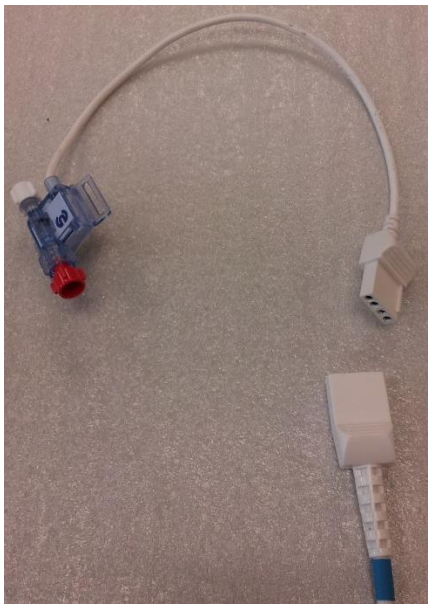
1. To connect the pressure transducers of the Data Acquisition System (optional) to the Scan Valve, cut the coloured heat shrink from the connector junctions near the end of the transducer cables as shown below.

2. Unplug the pressure transducers from the cable and save the transducer as a spare.



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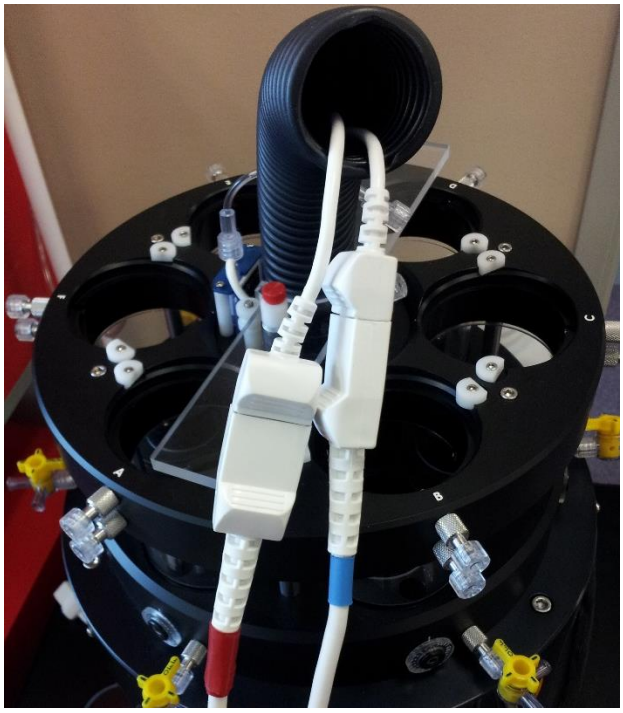
3. Plug the cables into the Scan Valve pressure transducer cables. Ensure the cables are plugged into the correct cable based on their colour coding (blue to inflow/lower transducer, red to outflow/upper transducer).



4. Connect the remainder of the Data Acquisition as shown in the schematic on the following page. See the Data Acquisition System User Manual for further information.

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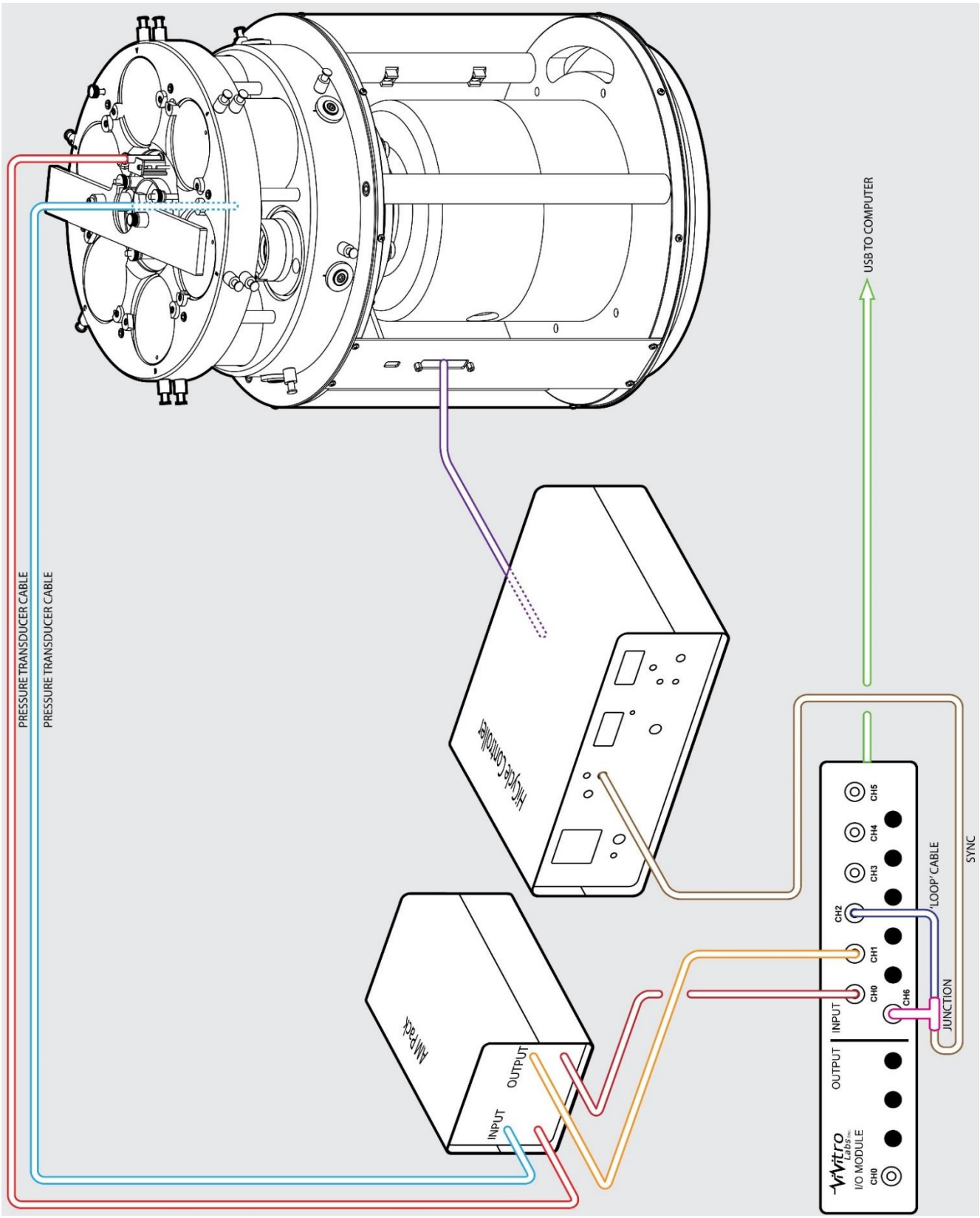

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4. System Setup

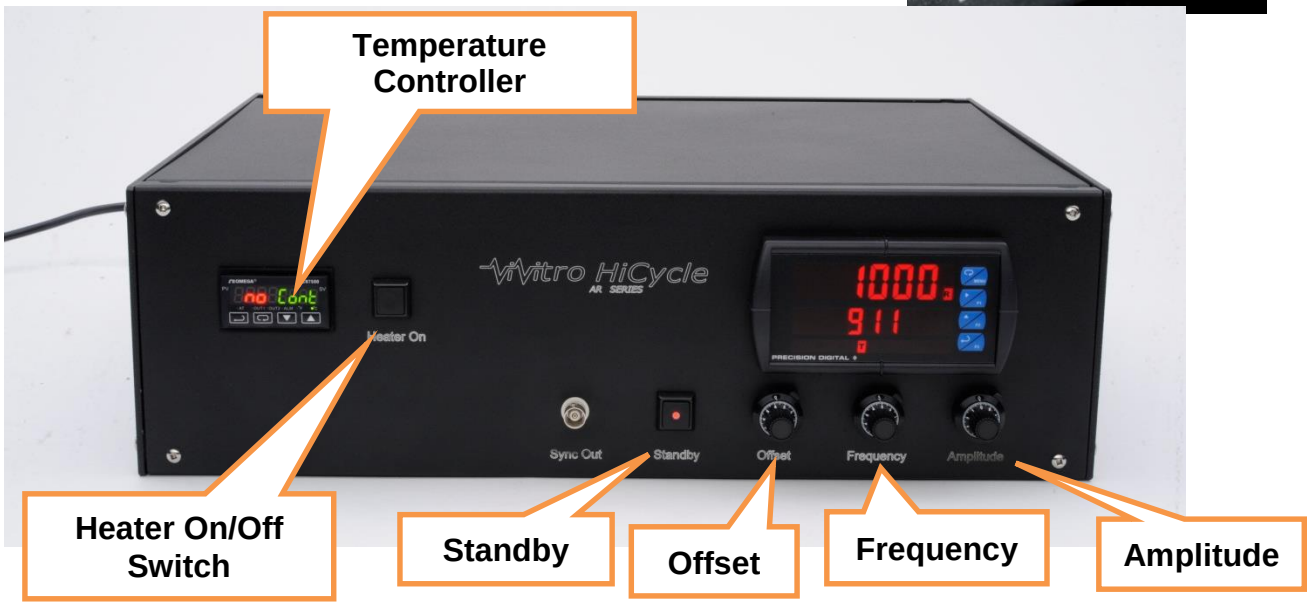
This section provides the user with

4.1 Controller Setup

Before switching the Controller power on:

1. Ensure all system cable connections have been made. See section 3.3 for details.
2. Set the Amplitude control to 0.0. If the dial will not turn, ensure the dial lock lever shown below is switched up for the unlock position.

   CAUTION: If the amplitude is not at 0 when the system starts, damage may occur at high amplitudes.
3. Set Offset control to about 5.0.
4. Set Frequency control to about 5.0.
5. Set the Temperature Controller to OFF
   (i.e. have the Heater On switch in the out position).
6. Set the Standby switch to the standby position (i.e. have the Standby switch in the pushed in position).



HiCycle Controller Front Panel

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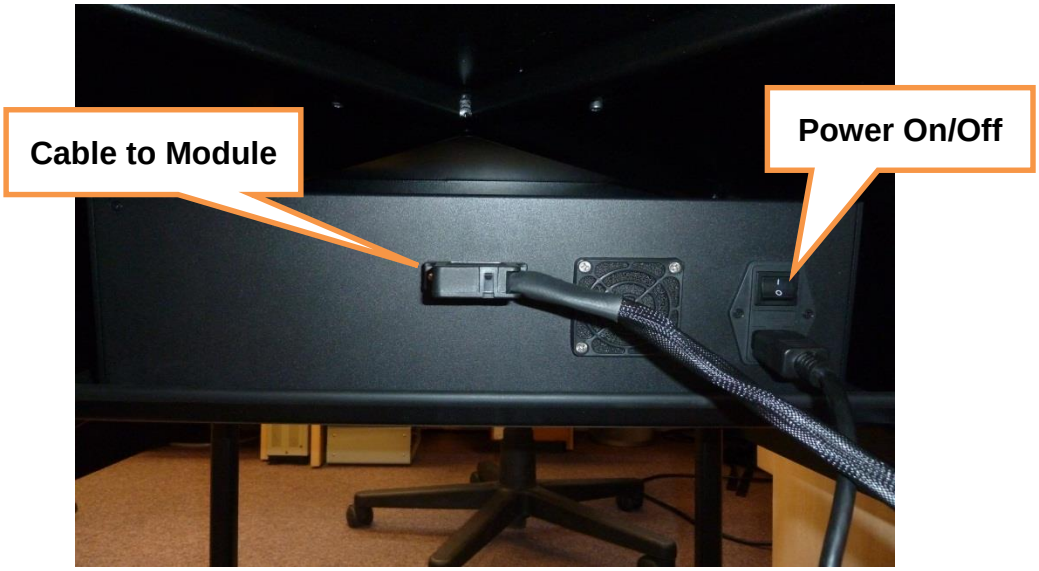
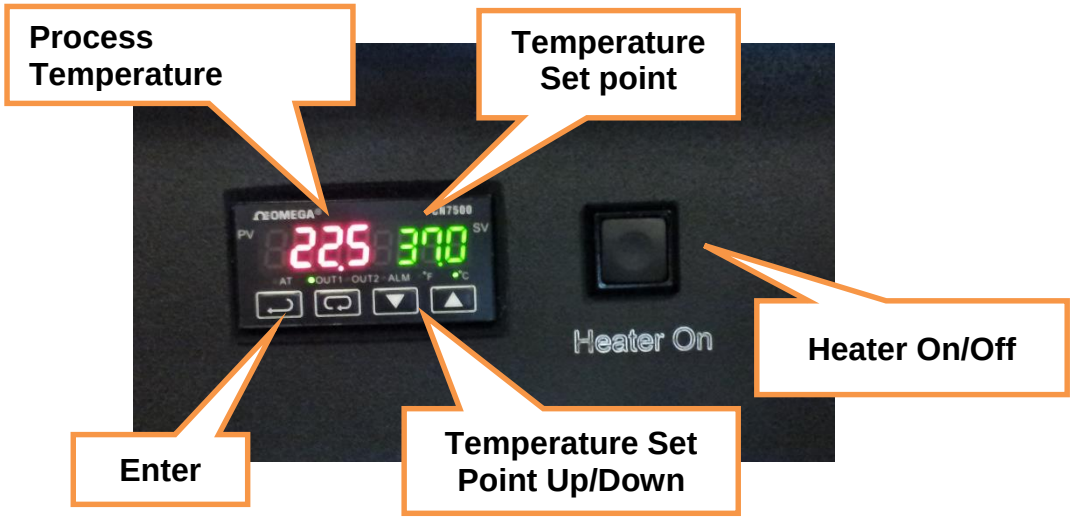


HiCycle Controller Rear Panel

7. Switch on the Controller using the rear panel power switch shown above.
   a) The Standby LED in the center of the Standby switch should be lit.
   b) The Module shaker should not be moving.

4.2 Heater Setup

1. Switch on the Omega Temperature Controller shown below by pressing the Heater On switch. The display will illuminate.



Omega Temperature Controller

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2. Set the temperature set point (displayed in°C) for the Module using the Up/Down arrows on the Temperature Controller.
3. Press the Enter button to set the temperature set point. The green LED to the left of the OUT1 text on the temperature controller will be illuminated when the system is heating.
4. The measured temperature should begin to rise toward the set point value (37.0°C typically) and the base plate of the test chambers should begin to warm.

NOTE: The system will heat more efficiently if the Module shaker is operating while heating due to circulation of the test fluid.

NOTE: DO NOT attempt to repair any problems arising from insufficient heating. If the temperature does not rise, discontinue use, power down the system, and notify ViVitro Labs.

If the temperature displayed on the controller varies by more than +/- 1.0°C when measured with a calibrated thermometer or when the temperature set point has been changed, the Auto Tune heating function must be set. Set the Auto Tune heating function as follows:

1. Press the Enter button. The display screen will read 'At oFF'.

2. Press the up ^ arrow to display 'At on'.

3. Press the Enter button to select 'At on'.
4. Press the Enter button again to return to the temperature display screen. The green LED to the left of the 'AT' symbol should now be flashing.

5. The system will heat until the temperature has stabilized at the set point. When the Auto Tune process is complete, the green LED to the left of the 'AT' symbol will turn off.

NOTE: When the temperature set point is changed, the Auto Tune function must be set again.

4.3 Piston Alignment Check

Alignment of the pistons within the test chambers is critical.

NOTE: If pistons are misaligned they may rub excessively against cylinder walls. This may affect the driver operation and/or cause damage to the Module. There is a minimum clearance between the piston and cylinder wall.

Improper alignment should be checked for as follows:

1. Remove all chamber windows (see section 6.1).
2. Turn on the Controller.
3. Ensure Amplitude is set to 0.0.
4. Press the Standby button to remove from standby mode.
5. Press the Start button.
6. Slowly turn the Amplitude knob to approximately 0.8.
7. Listen to each chamber carefully for a squeaking or chafing sound.
8. If any chafing sound is audible, see section 9.5 for alignment instructions.

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5. Controller Operation

The following sections describe the set up and use of the HiCycle Controller functions. Most Controller functions are managed by the Precision Digital Rate-Totalizer/Batch Counter Controller ("Rate Totalizer"). The Rate Totalizer buttons perform multiple functions, so the relevant function for each button press has been emphasized (e.g. Stop/**ent**/F3) in the procedure.

5.1 Frequency, Amplitude and Offset Controls

Amplitude, Offset and Frequency Controls

The Frequency, Amplitude, and Offset controls all include a dial lock switch. It is recommended that the dials be locked once the desired settings have been made to prevent the dial settings varying due to the vibration of the Module. See section 4.1.

The desired valve test frequency should be set before increasing the Amplitude setting. The Frequency dial sets the oscillating rate of the Module. The range of the Frequency setting is approximately 500 bpm (8.3 Hz) to 1650 bpm (27.5 Hz).

NOTE: It is not recommended to run the system at greater than 1080 bpm (18 Hz) due to loss of accuracy of pressure readings.

The Amplitude dial sets the drive level of the Module, which increases or decreases the peak to peak movement of the chamber pistons, and thus the pressures. The Amplitude control should be used as a tool for setting the chamber pressures, and not the absolute drive plate peak displacement.

The relationship between the Amplitude control number and the test chamber pressures or the piston displacement is not absolute but dynamic, as it depends on several factors, including:

- Frequency setting
- Offset setting
- Restrictor valve settings
- Forward and backward flow resistance determined by diameter, type, number, and mix of valves under test

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For a given HiCycle AR system, the Amplitude setting should be repeatable between test sessions if all other dependencies are also duplicated. The Amplitude setting should also be similar between HiCycle AR systems if all conditions are the same.

WARNING: The Amplitude must be reduced to minimum before Starting operation for the first time to prevent damage to the Module or test samples.

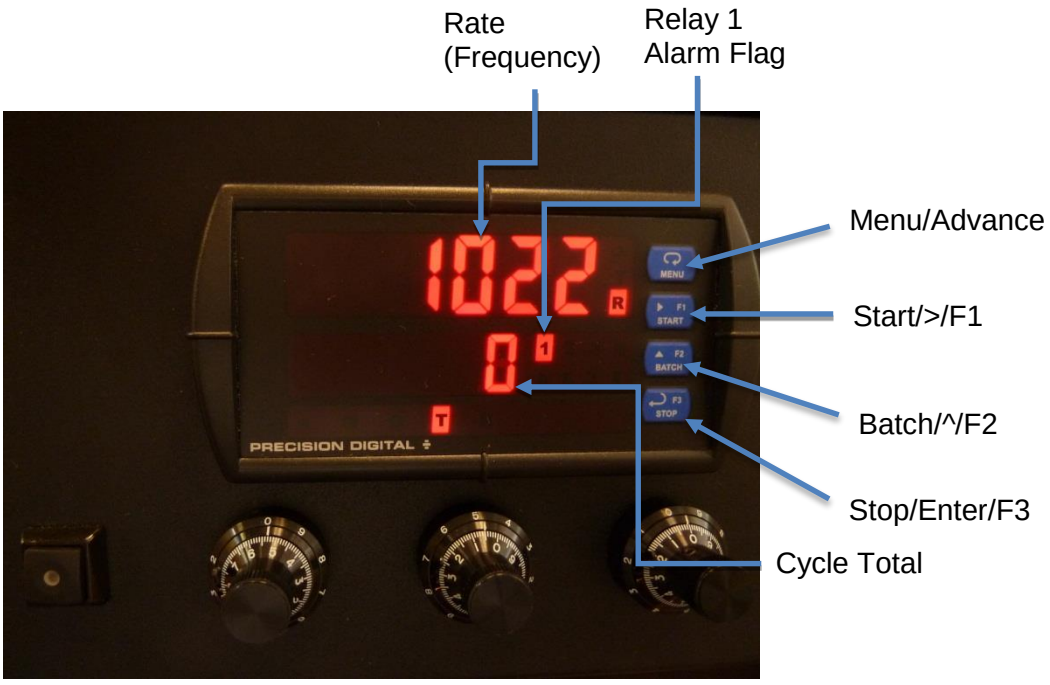
Once Module tuning has been completed (see section 8.5), it is still good practice to reduce Amplitude to 0 before restarting after a Pause or Stop event to reduce the possibility of transient overpressure events.

When stopping (or pausing) and restarting the HiCycle AR operation:

1. Pause or stop operation
2. Note the Amplitude setting
3. Reduce Amplitude to 0
4. Restart operation
5. Gradually increase Amplitude to the previous setting
6. Recheck pressures

The Offset panel control setting has some effect on the Frequency setting. The Frequency should be checked after adjusting Offset, and re-adjusted if required.

5.2 Operating Modes



Operating Mode Display

CAUTION: Keep hands and face away from the Module when starting.

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The Module can be Started, Paused, Resumed, or Stopped as described below:

1. **Start**
   - **Ensure the Amplitude dial is set to 0.0.**
   - Press **Start**/>/F1 to begin operating (The Relay **1** Alarm flag is displayed)
   - If the red LED on the controller Standby button is illuminated, press the Standby button to exit Standby mode.
   - Slowly increase the Amplitude dial to begin Module operation.

2. **Pause/Resume**
   - Press **Stop**/ent/F3 once to Pause operation. PAuSE will be displayed on the panel. Press **Start**/>/F1 to resume operation.
   - Operation may also be paused by pressing the Standby controller button (see below).
   - The red LED on the Standby button will be illuminated when in Standby or Pause mode.

3. **Stop**
   - Press **Stop**/ent/F3 a second time to Stop operation. StoP will be displayed on the panel. The red LED on the Standby switch will be illuminated when in Stop mode.
   -Press **Start**/>/F1 to resume operation.

While the Module is in running, operation will continue until the preset batch count is reached.
- The batch counter will be incremented and contain the count of the total number of batches since last reset.
- The Grand Total counter will contain the total count since last reset.
- The Total counter will be zeroed when operation is stopped.
- When the preset batch count is reached, the machine will stop and wait for the next Start command.

Relay 1 Flag turns off when Paused using **Stop**/ent/F3

*Rate Totalizer Display when Paused using **Stop**/ent/F3*

Relay 1 Flag remains when Paused using Standby switch

Rate Totalizer Display when Paused using Standby Panel Switch

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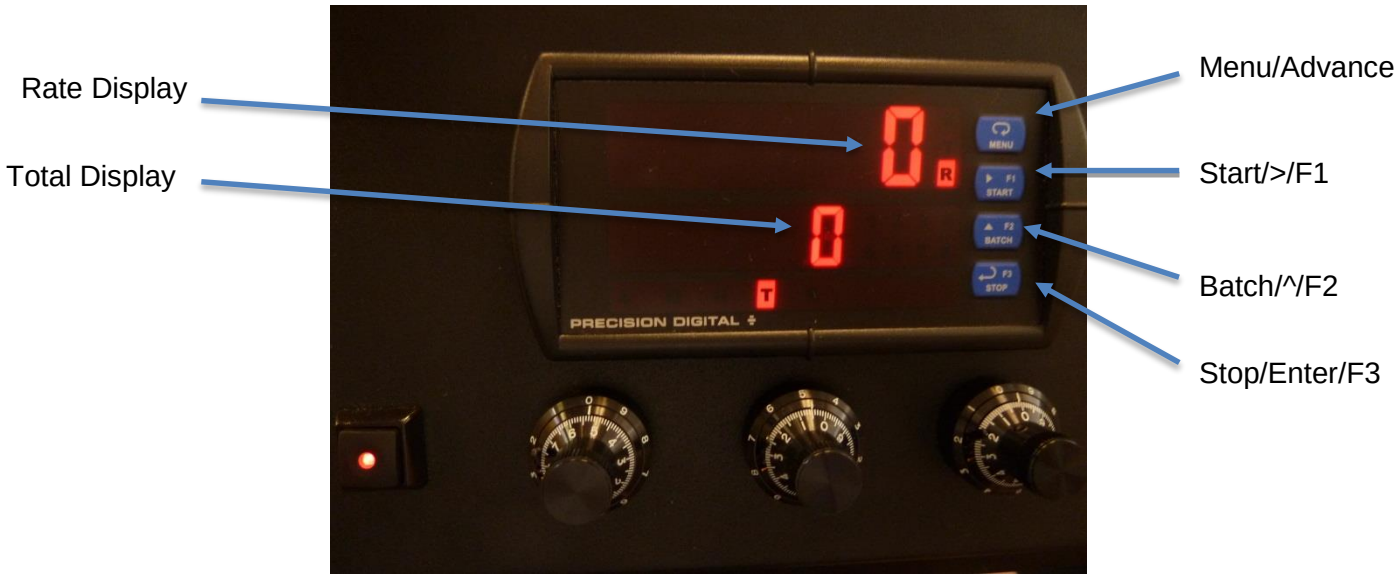
5.3 Default Rate Totalizer Display

The Default Display shown below is enabled on power up:

1. Upper display is **RATE** in cycles per minute
   - **R** flag is displayed adjacent to the rate field.
2. Lower display is nominally the current batch count **TOTAL**. The total counter will be zeroed when operation is stopped, then resumed. Pause mode or Standby mode will maintain the total count.
   - **T** flag is displayed below the total field.
   - The default lower display may be altered (see below).



Default Rate Totalizer Display

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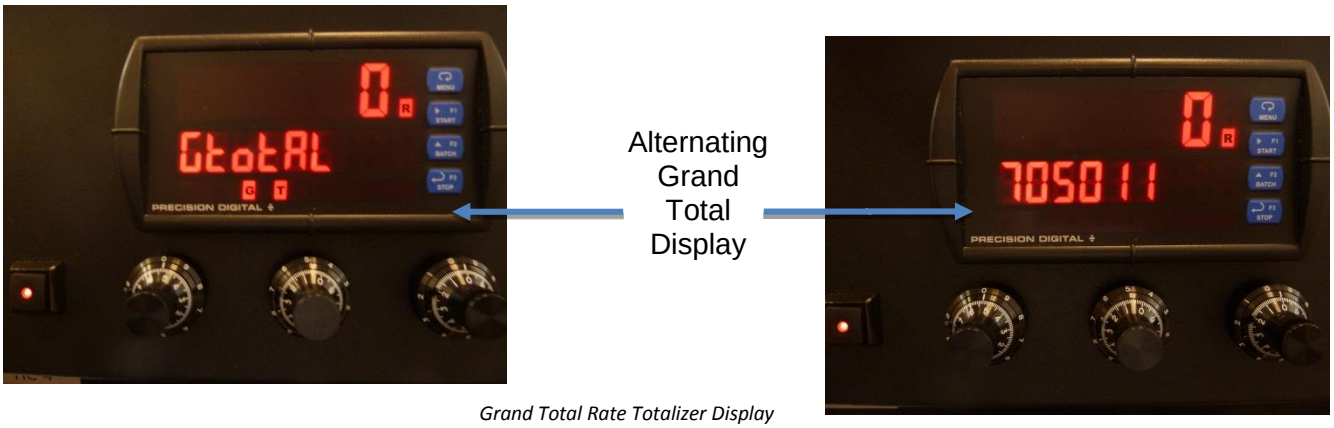
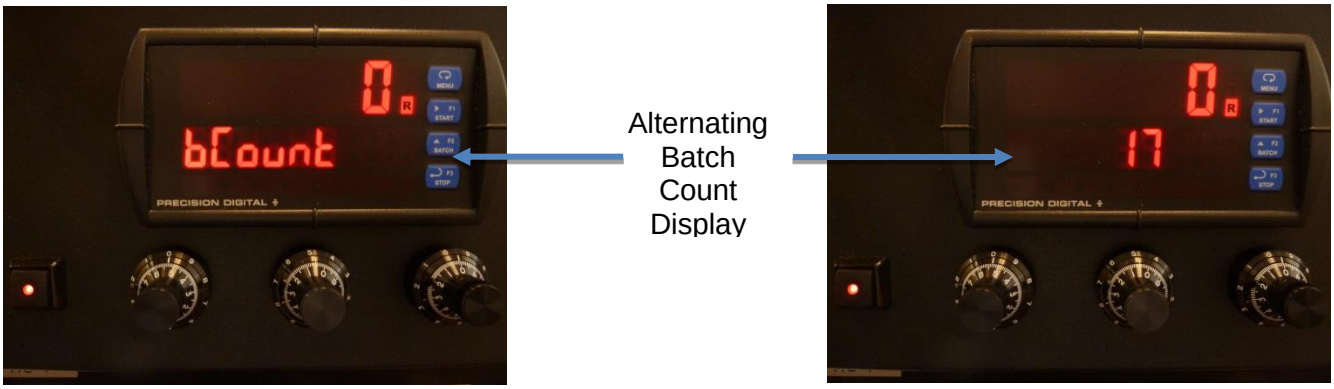
5.4 Alternate Rate Totalizer Displays



Grand Total Rate Totalizer Display



Batch Count Rate Totalizer Display

NOTE: The Controller must be in the stopped condition to change operation settings.

To display alternate count formats during Module testing:

1. **Grand Total Display**

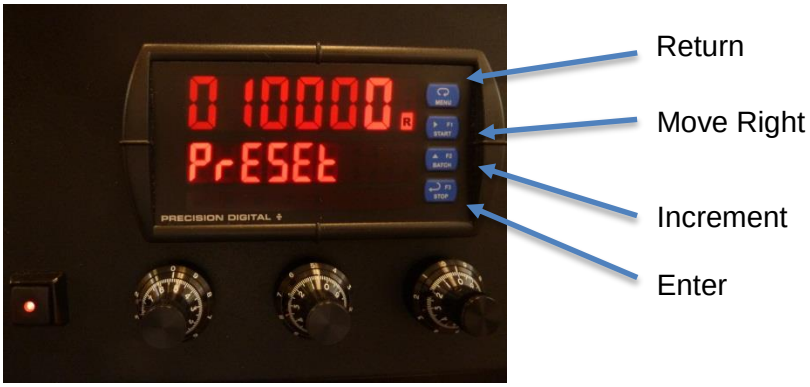
   The Grand Total counter displays the total count in thousands since the last reset. It can be initiated as follows:
   - Press Stop/**ent**/F3 once to display Grand Total in thousands.
   - **Gtotal** text is displayed momentarily, then the current Grand Total count in thousands.
   - **GT** flag is displayed below the count field.
   - The Grand Total count in thousands will be displayed during operation.

2. **Batch Count Display**

   The Batch Count counter displays the total number of cycle batches completed since last reset, based on the Batch Count Preset. Batch Count counter can be initiated as follows:

- Press Stop/**ent**/F3 to display current Batch count.
- **bCount** text is displayed.
- The Batch count will be displayed during operation.

5.5 Batch Counter Preset



Batch Counter Preset Value Entry

NOTE: The Controller must be in the stopped condition to change operation settings.

When in operation, the Module will continue to run until the Batch Counter Preset value has been reached. Once the Batch Counter Preset number of cycles is reached, the Module will stop and will not continue operation until the next Start command.

The Batch Counter Preset display can be initiated as follows:

1. From the default display, press Stop/**ent**/F3 three times to display batch count preset value in thousands.
   - **PrESET** text displayed momentarily, then the current batch Preset value.
2. Press **Batch**/^/F2 to display batch count preset in thousands.
3. Press Batch/**^**/F2 to increment current digit. The digit value wraps from 9 back to 0 and then repeats.
4. Press Start/**>**/F1 to move the edit field one character to the right. The edit field position wraps from the right-most character to the left-most character.
5. Press Stop/**ent**/F3 to enter the batch counter Preset value and return to the default display.

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5.6 Counter Reset Functions

Reset Mode Display

The counter display can be reset as follows:

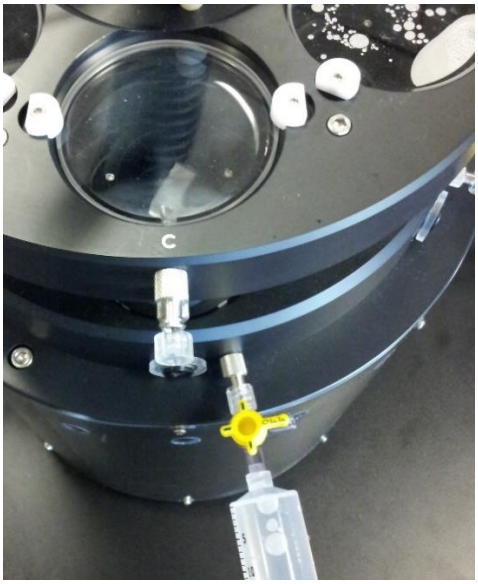
1. Press **Menu**/advance to enter menu, then Batch/**^**/F2 display **rESET**.
2. Press Stop/**ent**/F3 to select **rESET**, then press Batch/**^**/F2 until Reset Grandtotal (**rSt Gt**) heading is displayed with **rESET** on line 2.
3. Press Batch/**^**/F2 to cycle through:
   - **rESET t** (reset Total),
   - **rESET Gt** (reset Grand Total),
   - **rESET bc** (reset Batch Count),
   - reset H (reset max display – not applicable in this application),
   - reset L (reset min display – not applicable in this application),
   - reset HL (reset max and min displays – not applicable in this application),
4. Press **Menu**/advance to return to the default display without performing a reset.
5. Press Stop/**ent**/F3 to reset selected counter and return to main display.
6. Repeat steps 1 through 4 to reset another counter.

6. Module Operation

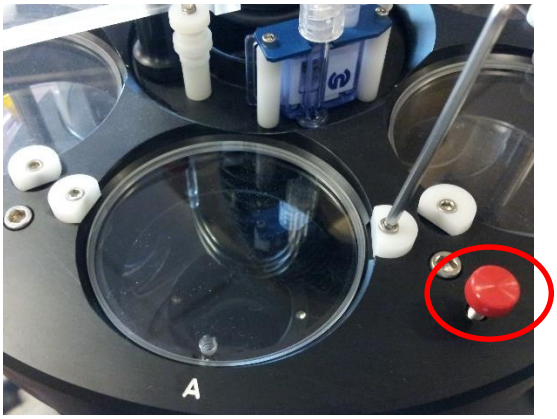
6.1 Chamber Window Removal

The windows can be removed with or without fluid in the chambers.

1. If the system is filled with fluid, close the head pressure stopcock to prevent spilling. If there is no fluid in the system, proceed to step 5.
2. Attach a syringe to the bottom drain port stopcock.



3. Open the chamber to atmosphere by unscrewing the top luer fitting cap.
4. Remove a small amount (or all if desired) of the fluid from the chamber using the syringe on the bottom drain port to prevent spilling during window removal.
5. Loosen the two bolts securing the white window retention clamps with a 3/32” driver and turn the white clamps until the window is unobstructed.



NOTE: One red thumbscrew is located on the top housing of the MODULE, unscrew this to use as a handle to remove the chamber windows.

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6. Screw the red thumbscrew into the pre-tapped hole in the chamber window. Lightly tighten until it just touches the bottom of threads.

7. Remove the luer cap from the top drain port if it has not yet been removed.
8. Gently pull window upward from chamber.

6.2 Chamber Window Insertion

Chamber windows can be inserted as follows:

1. Open the chamber to atmosphere by removing the top luer fitting cap.
2. Place the window on test chamber with the threaded hole facing upward. The threaded hole must be facing upward during installation or window removal will be very difficult. Be careful not to pinch the O-ring when pushing window into its seated position.
3. Turn white window retention clamps over the window and secure using 3/32" driver.

NOTE: Adjusting the window retention clamps after tuning the system will alter the operating pressures in the chamber. Ensure clamps are secure prior to tuning.

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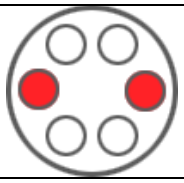
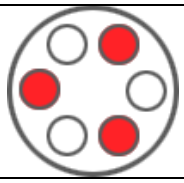
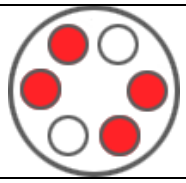
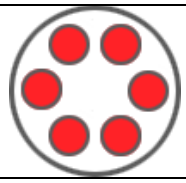
6.3 Mounting Test Samples

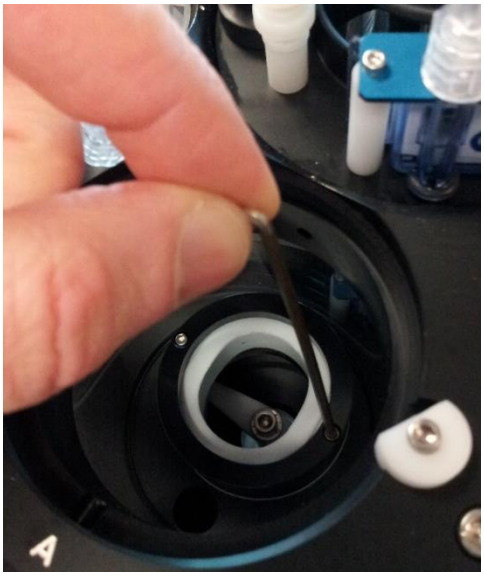
In order for the loading on the Module to be balanced, either 2, 3, 4, or 6 chambers should be used in the configurations shown below. Other configurations may be used but depend on t

It is recommended that the same valve type and size of sample be used in each test chamber. It may, however, be possible to achieve target test conditions with samples of one nominal size difference.

			
Two samples should be placed so that they are in diametrically opposite chambers.	Three samples should be placed in alternate chambers.	Four samples should be placed opposite each other.	Six samples loaded.

To mount a sample, follow these steps:

1. Remove chamber window (see section 6.1).
2. Unscrew the two bolts securing the piston cap using a 5/64” hex key. Remove the piston cap.



3. Fit sample into a molded silicon rubber ring specifically tailored to the sample’s geometry

NOTE: A jig is provided for molding valve mounting rings. These rings are common to all ViVitro Labs equipment. See Section 11 for a description of mold fabrication. Samples are usually mounted with their outflow side facing up to allow visualization of the outflow through the chamber window. See Section 12 for more information.

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4. Insert the silicone ring fitted with test sample into the piston cap ensuring the edge of the silicone ring is held securely underneath the retaining ring. Turn the assembly over to check the ring is concentric with the cap. Adjust if necessary.
5. Place cap assembly on the piston mount, aligning the two bolt holes with the threaded holes in the piston.
6. Check piston and cap are concentrically aligned.
7. Insert the two bolts into the cap and tighten using a 5/64" hex key.

NOTE: Take care not to scratch the TEFLON™ coating on the heater plate at the bottom of the test chambers.

6.4 Fluid Requirements

Sterile water or saline (0.9% by weight recommended) is a recommended test solution. An appropriate biocide to reduce the bioburden impact of contamination may be needed. Contact a supplier for a biocide recommendation and verify that it is compatible with the test chamber materials: Delrin™, Teflon™, Acrylic, Silicone rubber, Viton™ rubber and 316 Stainless Steel.

Approximately 1.8 liters of fluid (280ml per chamber) are required to fill the system.

6.5 Filling Chambers

All chambers must be filled with fluid when testing. Chambers without test valves must be filled with fluid so that air is not drawn through these chambers into the Scan Valve. See section 6.4 for fluid requirements.

NOTE: Before filling chambers with fluid through the open window ports, ensure that all ports on each chamber are closed or capped. When the windows are in place, filling with fluid can be completed and air removed from the chambers as follows:

1. Remove all chamber windows (see section 6.1).

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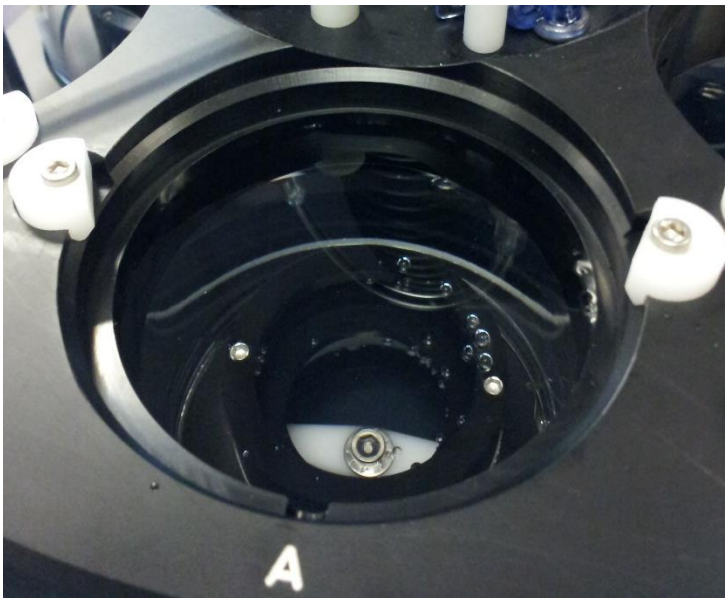
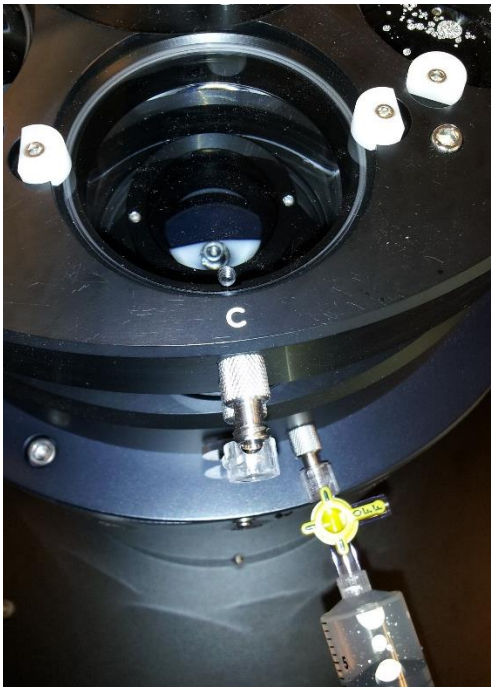
2. Fill each chamber with fluid ensuring that no leaks occur before continuing to the next chamber. Fill to approximately half way up the top chamber plate.



3. Using a non-abrasive instrument, dislodge any visible air bubbles from the chambers.
4. Remove the top luer fitting caps.
5. Replace the chamber windows and fasten in place (see section 6.2).
6. Attach a syringe filled with test fluid to the bottom drain port on the chamber. Open the stopcock and begin filling the chamber.
7. Continue filling until a small amount of water comes out of the open top luer fitting.



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8. Close the stopcock and remove the syringe.
9. Replace the cap on the top luer fitting.
10. Repeat steps 6 through 8 until all required chambers are filled.
11. Continue to section 6.7 to debubble chambers.

6.6 Pressure Head Assembly Operation

The Module should be operated under a positive head of pressure. The Pressure Head Assembly provided with the HiCycle system provides up to 65 mmHg of positive pressure head. The positive pressure in the system assists in sealing Scan Valve and helps to suppress the formation of bubbles caused by dynamic pressures which occur within the chambers.

1. Add test fluid to the Pressure Head Assembly and purge the air from the catheter line.
2. Turn the Scan Valve so that the transducer is set in between chambers. This connects all of the chambers through a passage in the scan valve.
3. Ensure the stopcocks connecting the Pressure Head Assembly to the chambers are open to allow passage of fluid from the Pressure Head syringe to the chambers. It may take a few minutes for the Pressure Head on the one chamber to bleed into the other chambers through the passage in the Scan Valve.

NOTE: Do not press the plug firmly into the syringe. This could prevent the fluid from freely flowing into the catheter.

NOTE: The level of fluid in the reservoir should remain constant. Evaporation with the stopper on the reservoir should be minimal, but check often and refill as needed. If the reservoir level falls rapidly, check the system for leaks.

NOTE: Before opening any valve chamber, the Pressure Head Assembly stopcock should be closed. This will prevent fluid being expelled from the chambers by the Pressure Head in the reservoir.

NOTE: When the scan valve pressure transducer is aligned with a chamber, the chamber is isolated. All remaining chambers are connected through the passage in the Scan Valve.

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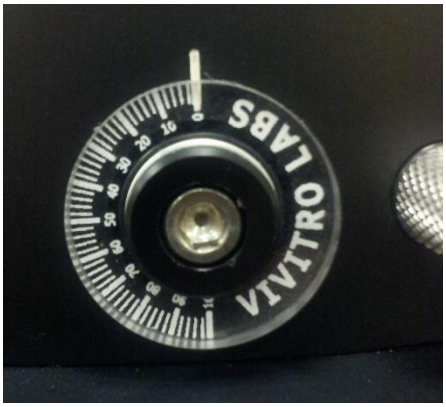
6.7 Debubbling Chambers

1. Before debubbling, ensure the scan valve pressure transducer is not aligned with a chamber. The position of the scan valve should be as shown below.

2. Ensure that all three chamber luer fitting ports on all chambers are closed.
3. Turn all flow restrictors to 0%.



4. Turn the controller on and press the **Start**/>/F1 button and press the Standby button to release from Standby mode.
5. Increase the amplitude slightly until the piston caps are visibly moving.
6. Using the Frequency dial, cycle through the full range of frequencies until bubbles are no longer being dislodged.
7. Press the Standby button to pause the Module and turn the Amplitude to 0.
8. Attach a syringe containing approximately 10 ml test fluid and no air to the bottom stopcock and open the stopcock.
9. Ensure the pressure head stopcock is closed.

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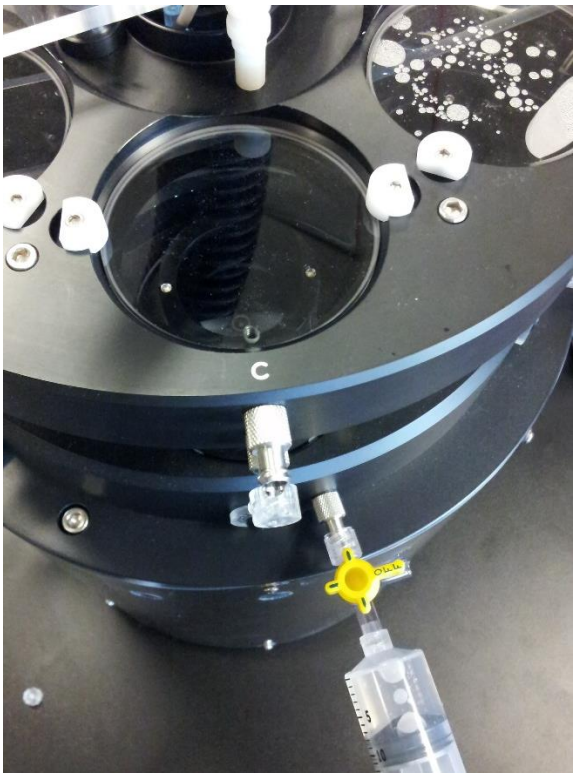
10. Remove the top luer fitting cap to open chamber to atmosphere.
11. Withdraw approximately 25 ml fluid so that all of small bubbles beneath the window are captured by the drawn in air.



12. Slowly push the plunger of the syringe in to expel all air through the upper port. Be careful to not introduce any new air bubbles to the chamber from the syringe.
13. Replace the luer fitting cap on the top port.
14. Close the stopcock on the bottom drain port and remove the syringe.

NOTE: The above steps may need to be repeated several times before all chambers have a minimal amount of air bubbles. The debubbling process may be shortened by attaching and filling the Pressure Head reservoir (see section 3.3). With the Scan Valve NOT aligned with a chamber, air bubbles can be expelled by the pressure head through the chamber when the top port is opened.

6.8 Draining Chambers

1. With the viewing window on, and the pressure head stopcock closed, attach an empty syringe to the bottom drain port stopcock on the chamber.
2. Open the bottom drain port stopcock.
3. Open chamber to atmosphere by removing top luer fitting cap.
4. Begin emptying the chamber using the syringe.

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5. When the syringe is full close the bottom drain port stopcock.
6. Remove the syringe and empty it.
7. Continue and repeat steps 1 through 6 until all chambers are empty.
8. With windows removed (see section 6.1), residual fluid can be removed using a syringe.

6.9 Scan Valve Operation

The Scan Valve is supplied with two pressure transducers installed to measure inflow and outflow pressure of each valve. The vertically mounted pressure transducer on the Scan Valve should be used as a guide for alignment with the index mark on each chamber.

NOTE: When pressures are not being monitored, the Scan Valve should be set between chambers as shown below. This will eliminate cycling pressures from acting on the transducers, extending the transducer life.

Scan Valve aligned between chambers while not monitoring pressure

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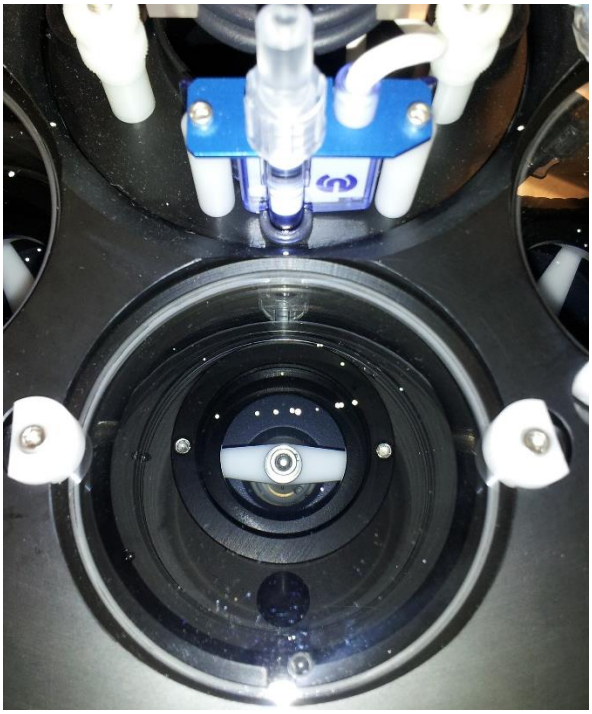

Scan Valve aligned with chamber to monitor pressure

The tension between upper and lower Scan Valve elements has been set at ViVitro Labs to give minimum friction while still sealing. The three knurled white tension adjustment knobs on top of the Scan Valve have been factory set. Normally, no adjustment of the tension should be necessary.

It is possible, that as the unit is warming from room temperature to the set-point temperature and before a Pressure Head is applied to the unit, there may be some initial seepage of test fluid from the upper or lower Scan Valve elements. This can be removed with an absorbent cloth. If it occurs, seepage should cease when the Scan Valve elements are pressurized and the Module temperature is stable.

The Scan Valve should be turned using both hands on the handle. If the Scan Valve is difficult to turn, friction may be reduced by turning each of the three knurled white thumb nuts on the top of Scan Valve anti-clockwise a few degrees. If the Scan Valve assembly seems locked it may be due to mis-seating of the tapers in the upper and/or lower Scan Valve elements of the assembly. They may be unlocked and re-seated as follows:

1. Turn each of the three white knurled adjustment knobs anti-clockwise approximately 30°.
2. Push down simultaneously on the ends of the three (3) threaded draw rods to unlock lower element of Scan Valve.
3. Place fingers underneath upper element and pull upwards to unlock.
4. Tighten knurled knobs evenly for free movement of Scan Valve.

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If the Pressure Head does not appear to be reaching all of the chambers (i.e. fluid does not seep out of any open stopcocks when the pressure head is connected and open), the Scan Valve may be over tightened. The Scan Valve may be checked for over tightening as follows:

1. With no fluid in the test chambers, attach a manometer to the top luer fitting.
2. Connect a syringe full of air to the bottom luer fitting of the same chamber.
3. With the scan Valve oriented between chambers, attempt to pressurize the chamber to 100 mmHg with the syringe.

4. If the chamber holds pressure with a leakage rate less than approximately 10 mmHg in 10 seconds, loosen the knurled white thumb screws on top of the scan valve anti-clockwise a few degrees each and repeat steps 1 through 4.

After loosening the Scan Valve, monitor the system for small leaks in the instance that the Scan Valve is over loosened. If leakage occurs, tighten as described in the directions above.

6.10 Safety Caps

As the Scan Valve is rotated away from each chamber, low frequency transient pressures, distinct from chamber pressures, may be generated and can damage the pressure transducers. To attenuate these pressures, the transducers are shipped with a unique Safety Cap installed (see image below).

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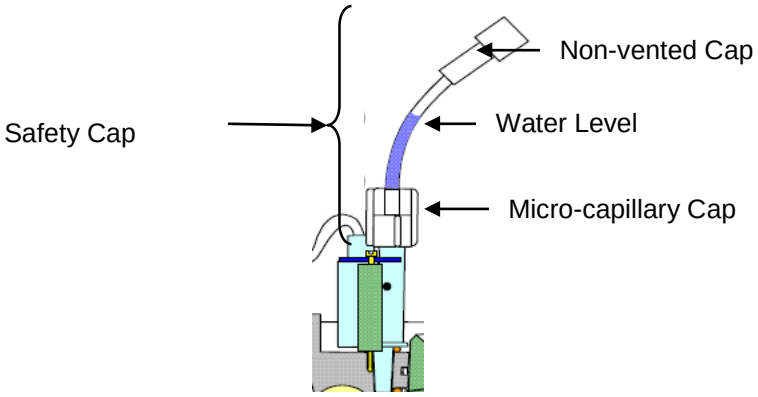

Pressure Transducer Safety Cap

NOTE: The pressure transducers in the Scan Valve should only be capped with the Safety Caps provided.

The Safety Cap includes a micro-channel and an entrapped air volume to suppress low frequency pressure transients without compromising high frequency pressure fidelity of the transducer system.

To be effective, the Safety Cap should contain an entrapped air volume and can be primed as follows:

1. Position the Scan Valve to be aligned with a chamber that is open to atmosphere.
2. Remove the transducer's Safety Cap.
3. Remove the non-vented cap from the Safety Cap.
4. Attach an air-filled syringe to the Safety Cap connector (if necessary, remove fluid from catheter line using the syringe).
5. Place the micro-capillary cap in a container of distilled water.
6. Use the syringe to draw fluid through micro-capillary cap until catheter line is half filled with fluid. If the micro-capillary is blocked, the Safety Cap is not functioning properly and should be discarded and replaced with a new assembly.
7. Remove the syringe and secure the non-vented cap to entrap the air volume.
8. Place the Safety Cap on the transducer.

The Safety Cap on the lower Scan Valve pressure transducer can be removed as follows:

1. Align the Scan Valve so that the lower Safety Cap is visible in between chambers.
2. Insert a flat blade screwdriver in between the chambers.
3. Place the blade of the screwdriver in the grooves of the Safety Cap fitting.

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4. Gently use the screwdriver as a lever against the chamber and turn the Safety Cap anti-clockwise to loosen it from the pressure transducer. If the positioning is making this difficult, reposition the Scan Valve and repeat.
5. The Safety Cap can be replaced by threading it onto the pressure transducer using the technique described in step 4 above and turning the Safety Cap fitting clockwise to tighten. BE CAREFUL NOT TO OVERTIGHTEN.

6.11 Debubbling of Pressure Transducer Channels

Any air bubbles in the pressure transducers or in the channels from the transducers to the test chambers will compromise the fidelity of the pressure records. Hidden bubbles may be evident from the oscillatory character of the pressure record (see image below). The best practice to ensure pressure measurement accuracy is by removing air bubbles in the following manner:

1. Close the Pressure Head stopcock.
2. Turn the Scan Valve to align with a chamber.
3. Attach a fluid filled syringe to the bottom stopcock.
4. Remove the Safety Cap from the transducer.
5. Flush the transducer and lines by injecting fluid into the chamber using the syringe.
6. Replace the Safety Cap.

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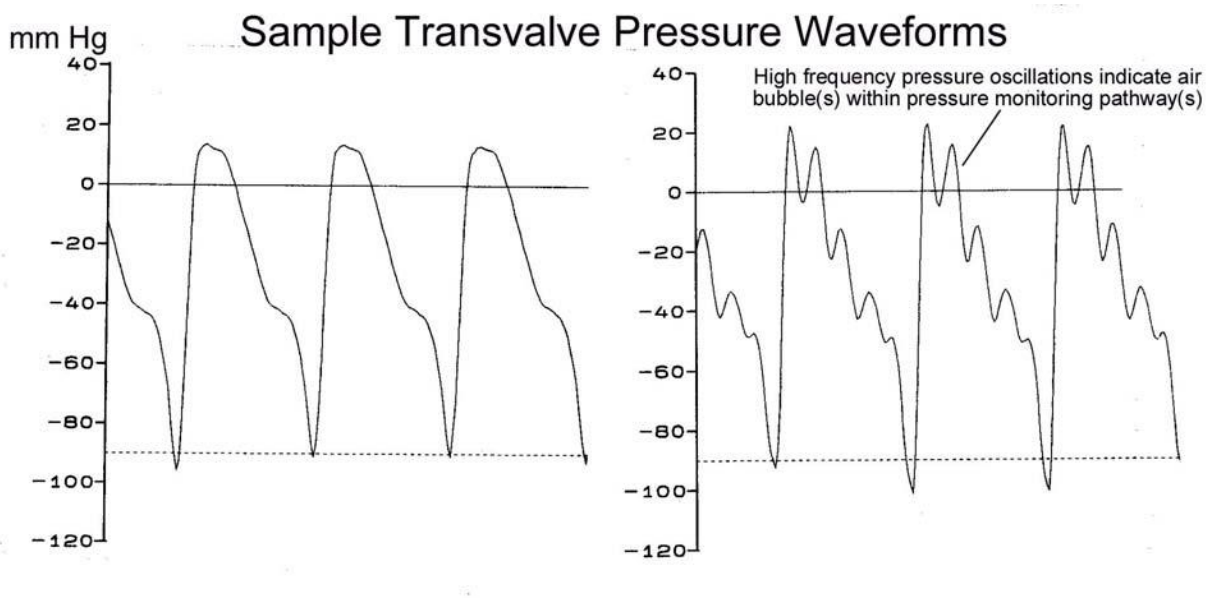
7. Open the Pressure Head stopcock.

NOTE: An alternate way to de-bubble pressure transducers is by removing the Safety Cap and then turning Scan Valve so that the Pressure Head forces fluid through the transducer and pressure channels. The pressure transducers in the Scan Valve should only be capped with the Safety Caps provided.

The Safety Cap on the lower transducer can be removed/tightened by using a flat blade driver as described in section 6.10. The driver should be inserted horizontally between valve chamber cylinders and carefully levered against a cylinder so that the blade moves the serrated cap clockwise to tighten or anti-clockwise to loosen. BE CAREFUL NOT TO OVERTIGHTEN.

Below is a representation of a normal pressure waveform (left) and a pressure waveform that has excessive noise due to air bubbles in the pressure transducer port (right).



6.12 Compensation Spring Adjustment

In normal operation there will probably be a mean downward force on the driver caused by two factors:

1. Hydrostatic Pressure Head from reservoir.
2. Greater mean closed transvalve pressure than open transvalve pressure.

This asymmetrical load causes the mean position of the shaker armature to change from the unloaded mean position. This reduces shaker stroke capability and can be avoided by using spring compensation. A spring in the center of the shaker should be adjusted to compensate for asymmetrical loading:

1. Turn Controller to Standby mode.
2. Close the pressure head stopcock.

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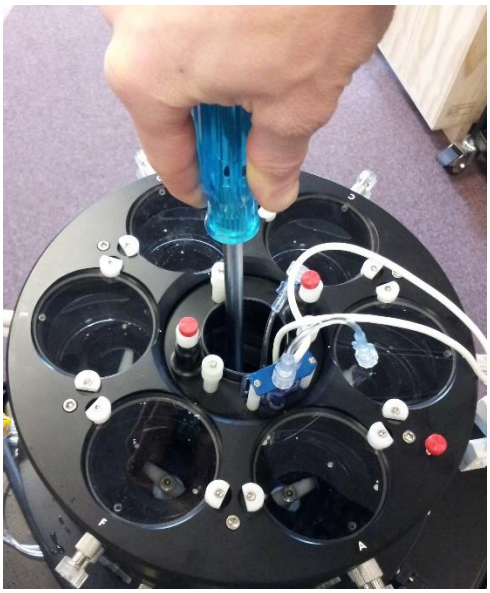
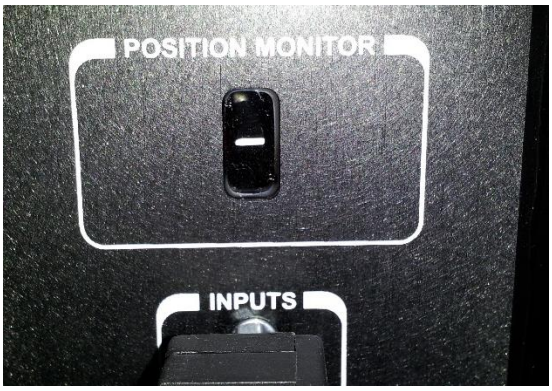
3. Remove the scan valve handle.
4. Release spring compression by turning nut in center of Module by turning anti-clockwise with the socket driver provided in the accessories kit.



5. Note alignment of Position Monitor indicator relative to the center of the Position Monitor window on the Module.



6. Adjust Hi-Cycle to dynamic operating conditions.
7. Rotate nut so the Position Indicator oscillates about the center of the Position Monitor window.

NOTE: Adjustment of the compensation spring may change shaker peak-to-peak amplitude. Therefore, transvalve pressures may need to be re-adjusted to achieve target conditions after adjustment of the compensation spring.

NOTE: When the driver is at rest for extended periods of time, the compensation spring compression should be released to maintain the relaxed condition of the driver suspension mechanism.

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7. HiTest Software Introduction

HiTest is designed for periodic monitoring, acquisition and analysis of data generated during testing of heart valves over several million cycles. The software consists of two primary processes: waveform acquisition and statistical calculations based on the acquired waveforms.

HiTest Hardware Requirements

The HiTest software application is designed to run on the following minimum hardware specifications:

- LCD monitor capable of 1600 x 900 resolution
- High throughput video card
- I/O Module
- 4GB RAM
- 320GB Hard drive
- Quad core processor 2.66Ghz or better

Software Requirements

For the software to run smoothly the following software is required to be installed on the computer:

- Microsoft Windows 7 Professional

.NET 3.5 framework

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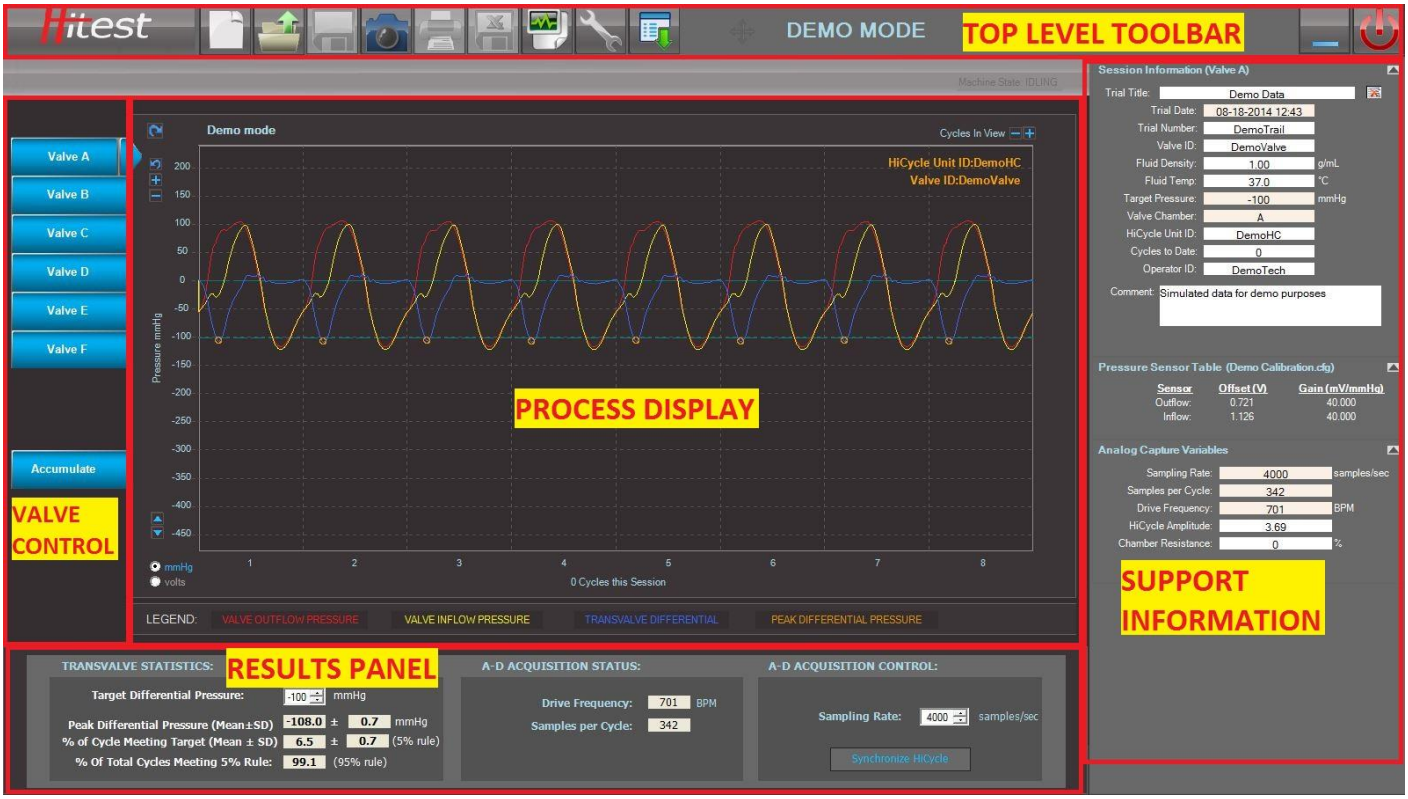
7.1 Software Interface Orientation

Below is the home screen of HiTest.



HiTest – Screen Layout

7.2 Top Level Toolbar

HiTest Top Level Toolbar

Standard controls in the top level toolbar are:

New Trial Resets in preparation for a new valve trial.

Open File Brings up a standard open file dialog window to allow users to select a valve test file for review.

Save File

Saves a valve test file (.tst file) for the currently selected valve in focus – its statistics, means and its latest raw pressure buffer.

Clicking the Save button saves the status of the latest acquisition to a test file (.tst). This allows users to record a data snapshot of the valve's behavior for later review.

Typically, users doing extended testing of a valve will periodically (daily or even weekly) accumulate several cycles and then save a test file for the latest accumulation to record the valve state at that time.

Snapshot

Captures a screen snapshot (.jpg file) of the acquisition graph. To capture the full HiTest window, hold the shift key down while clicking the icon.

Print

Creates a report (.html file) of current conditions and mean results for the currently selected valve being tested.

Save .csv File

Saves most recent valve test statistics, means and raw pressure data to a CSV (comma separated value .csv file) type text file for ready access.

Averages and A-D voltage data for a single A-D period are saved.

Append

Appends a current state entry to the (.log) file for the current test.

The log file name is built from the trial date. Live acquisition is interrupted only briefly and the user is notified if writing to the log file fails due to an error.

A hidden log is kept for every HiCycle session as a means of auditing events and user actions for regulatory and troubleshooting purposes. This is

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a background process and there is no indication at the time these entries are made. This log name is simply a time and date stamp (e.g. 10-17-09 15-04.log). These log files are kept in the C:\Program_Files\ViVitro_Labs\HiTest\Files\Logs folder and should be archived or cleared out periodically when they are no longer likely to be referenced.

Calibrate

Interrupts Live Running mode and starts the calibration sequence. Users can manually edit or re-measure pressure sensor gains and zero offsets to update the calibration file (.cfg file) for the connected HiCycle Unit.

All Menu

Access to all features and information about HiTest including:

- Graph sync signal
- Open Excel on .csv file save option
- Loading calibration files
- User manual download

Reposition

Clicking this icon and dragging will reposition the HiTest session window.

Minimize

Minimize the software window to the Windows toolbar.

Exit

Closes the application.

NOTE: Most controls on the user interface have tooltips that give a brief explanation of usage whenever the mouse is hovered over the control.

NOTE: All files saved from the software are saved to the Files folder of the software directory. (e.g. C:\Program Files\ViVitro Labs\HiTest\Files)

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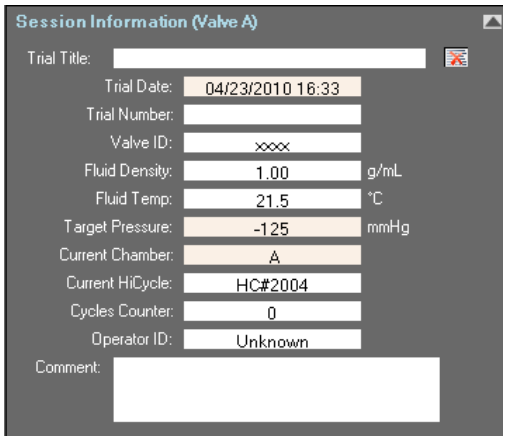
7.3 Support Information Area

The Support Information Area hosts key information regarding valve and trial readings/results for your sessions.

NOTE: This area depicts the information for the selected valve according to the Valve Controls on the right of the screen.

Collapses the selected data area.
Opens the selected data area.



Session Information:

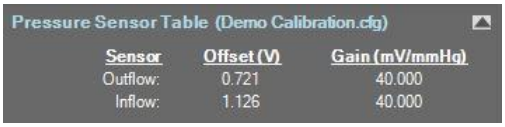
Operators are expected to complete each field to ensure that each trial and valve can be individually characterized.

Most fields are editable, however, the Target Pressure value is taken from the Target Differential Pressure toggle button field for the selected valve chamber in the TRANSVALVE STATISTICS area at the bottom of the screen.

Trial date automatically loads the current date and time when accumulation is started.

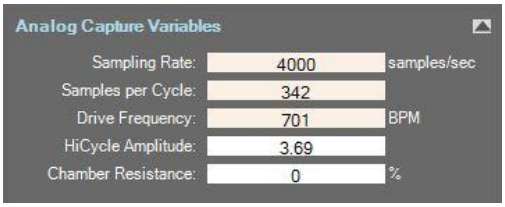
While creating data for each valve note that the information remains the same in each field. The operator is to manually change the Valve and Trial details according to what is required.

Clears the information in the fields of Trial Title; Trial Number and Valve ID.



Pressure Sensor Table:

Displays data extracted from the currently loaded calibration file presenting calibration values. Displays active calibration file name.



Analog Capture Variables:

Displays the mean values for the performance of the trial. HiCycle Amplitude and Chamber Resistance fields are editable and require the operator to enter settings read from the Controller and Module respectively.

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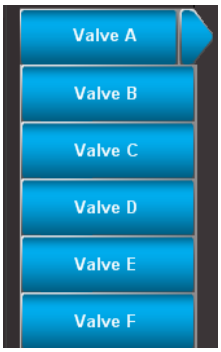
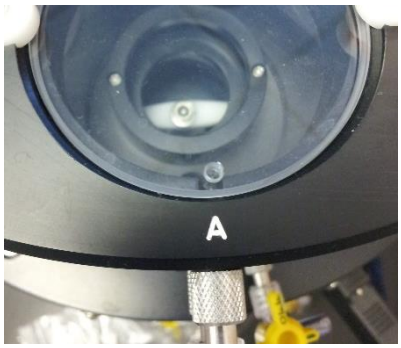
7.4 Valve Controls

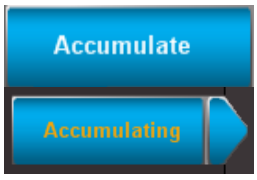
Toggle through valve data by selecting buttons Valve A to F. Valve data is displayed on the screen updating all fields and process display graph for the valve selected. One valve can be viewed at a time.





NOTE: Operators are advised to correspond software lettering with the HiCycle chamber's reference letter. When switching between different valves ensure the Scan Valve on the HiCycle Module is collecting data from the corresponding valve chamber.

7.5 Accumulating & Resume Live Control



Accumulating

Selecting this will bring up a new window requesting a number of cycles you would like to run in your trial. This is used to demonstrate a direct segment of the trial's test by accumulating all associated data. Once the trial is run through the option to save the data as a .csv file appears. The accumulated data will then appear within the process display.

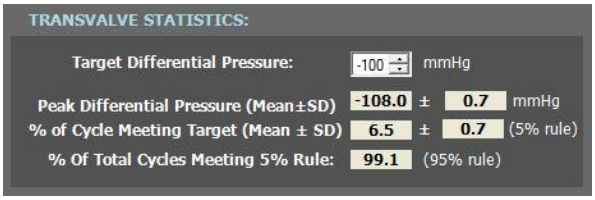
Selecting the Accumulating button while the accumulation is in process will abort the activity.

Resume Live

To resume the live data view when viewing accumulated data, select this button.

7.6 Results Panel

The results panel hosts running statistics regarding pressure loads and A-D Acquisition. It allows operators to adjust the way live pressure data is acquired.



Transvalve Statistics

Manipulate the Target Pressure Boundary here to affect the Session Information data.

Live mean differential pressure statistics tracking valve performance during extended testing are displayed.

As a trial is running the % rules will display as each cycle is complete. The Peak Differential Pressure is displayed in this panel.

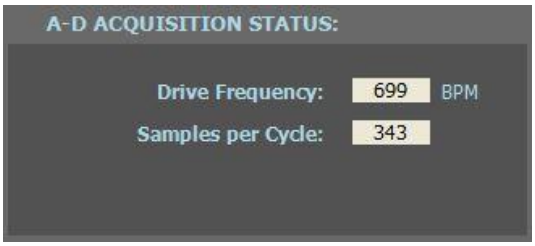
NOTE: The target transvalve pressure threshold is signed negative to indicate it is the pressure across the closed valve. This target pressure load threshold sets the criterion by which two important results needed for ISO 5840:2013 compliance are calculated:

95% and 5% Rule

- Percent of cycle reaching or exceeding the target pressure (5% rule)
- Percent of cycles that exceed the target pressure threshold for >= 5% of the cycle (95% rule)

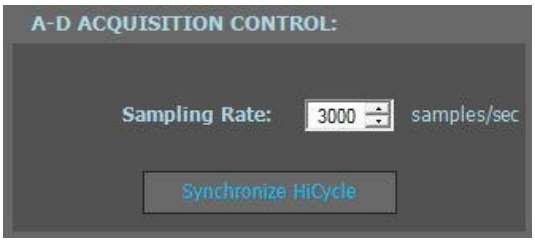
ISO 5840:2013 Appendix O.7 states that "verification that targeted pressure across the closed valve were attained for at least 5% of each cycle during at least 95% of the test cycles" should be performed. This is shown in the % All Peak Pressures Meeting Target field.

These results are shown in the printed report for the trial. Also shown is the mean Peak Differential Pressure value and deviation.



A-D Acquisition Status

Displays the trial's drive cycle rate and how many samples are run per cycle.



A-D Acquisition Control

Manipulate the A-D sampling rate by toggling up or down to the desired value. The Synchronize HiCycle button should be clicked when the Sampling Rate has been adjusted and when the Frequency has been adjusted on the Controller. The Sampling Rate can be adjusted between 1200 and 4000 samples/second. A sampling rate of 4000

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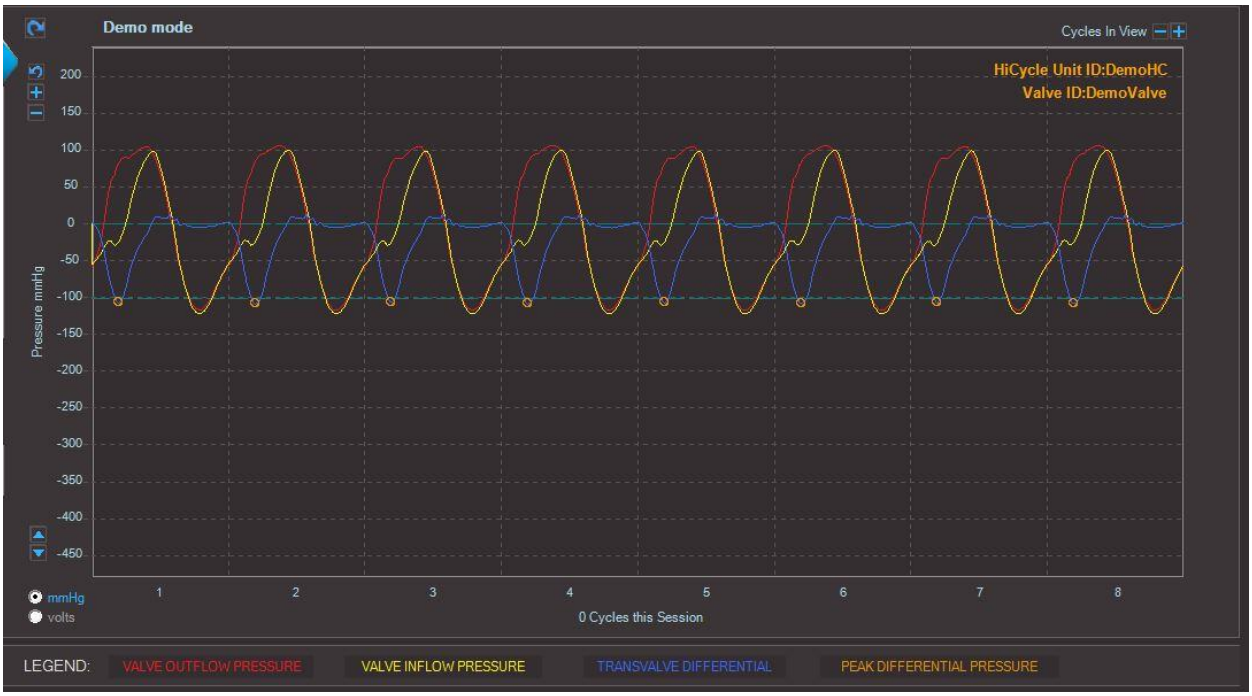
samples/second is recommended. If there is a mismatch between the sampling rate on the controller and in HiTest, increase the sampling rate and click Synchronize HiCycle.

NOTE: A software timer whose default rate is 2 Hz determines how often the A-D card is instructed to perform an update.

NOTE: The acquisition rate (default 4000) defines how quickly data is digitized for each of 3 channels in a simple outflow/inflow/sync system and thereby the resolution of the digitized waveform.

7.7 Process Display



The Process Display shows the information being read by the system including:

- Most recent pressure sensor values for most recent ADC analysis
- Multiple valve cycles
- A pair of horizontal lines show the 0 pressure or voltage axis and the target pressure threshold against which valve performance is measured
- The calibration file being used and the date and time it was last updated
- A legend bar indicates the meaning of the various colored graph traces. These can be turned on/off by clicking the text in the legend to simplify the display.

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- RED line – Valve Outflow Pressure
- YELLOW line – Valve Inflow Pressure
- BLUE line – Transvalve Differential
- ORANGE circle – Peak Differential Pressure

NOTE: During the free-running acquisition phase ('Idling'), voltages measured by the A-D card are rendered as traces in the graph panel every ½ second – the default A-D period or cycle. During this period a series of cycles result in the repetitive waveforms on the graph.

NOTE: Except in test file review mode when the graph is static, the pressure graph is redrawn according the A-D cycle period which defaults to 2 Hz.

The graph can be manipulated by the following controls:

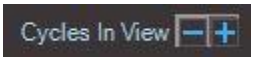
Refresh – Refreshes/reloads the data on the pressure graph

Restore – restores the graph to the default viewing scale

Increase/Decrease (zoom) – manipulates the scale of the pressure axis

Up/Down – enables the operator to reposition the graph data on the screen by adjusting the Y axis

mmHg/Volts – enables the operator to toggle between mmHg and volts, adapting the Y axis units, depending on what measurement reading is desired



Cycles in View – Increases/decreases the cycles in view on the process display between a minimum of 3 and a maximum (and default) of 8 cycles.

7.8 Help

The user manual can be downloaded by selecting Browse User Manual under the All Menu button. The user will be directed to a download link on the ViVitro Labs website. For any further questions, contact ViVitro Labs directly at www.vivitrolabs.com.

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8. HiTest Software Operation

Before using HiTest, verify that the Software and Data Acquisition System (optional) are functioning peoperly:

1. Start the computer and logon.
2. Prepare the HiCycle Unit for operation (see Section 4).
3. Verify the software installation and the hardware function by:
   - Positioning the HiCycle Scan Valve to a desired chamber.
   - Set HiCycle Controller to standby and temperature control to off.
   - Select the HiTest icon on the desktop to launch the HiTest software.
   - Select the factory settings configuration file – HiCycle.cfg.
   - Verify that the “Drive Cycle Rate” displayed under A-D Acquisition Status within the software corresponds approximately to the DRIVE FREQUENCY value displayed on the front panel of the HiCycle Controller.

The system is now ready to monitor the operation of the HiCycle System using HiTest Software.

8.1 Test Preparation

Before beginning calibration and testing, prepare the system as follows:

1. Prepare the HiCycle Module for operation with valves as per section 4.
2. Position the Scan Valve to the desired chamber.
3. Prepare the HiCycle Controller:
   - Turn on the controller
   - Drive set to “Standby”
   - Amplitude set to Zero
   - Temperature is Off
   - Verify that all connections are in place
4. Commence HiCycle Controller and Module operation:
   - Turn the heater on (temperature will rise to the preset, e.g. 37°C)
   - Press **Start**/>/F1
   - Take the Controller out of Standby mode
   - Increase the amplitude on the Controller to the desired level
5. Start the computer and logon.

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6. Open Instacal.
7. Right click and select Remove Board.
8. Click OK.
9. Click Refresh.
10. Click OK.
11. Right Click and Select Configure.
12. Set the ADC Settling Time to 10us.
13. Click OK.
14. Close Instacal.
15. Double click on the HiTest icon located on your desktop.
16. Select the desired calibration configuration file.

NOTE: The "Demo Calibration" file installed in "C:\Program_Files\ViVitro_Labs\HiTest\Files" contains factory calibrations for the pressure measuring system. These should be sufficiently accurate for exploratory use of the HiCycle System.

NOTE: A checksum test will alert the operator should a configuration file become corrupted. If this occurs either substitute a backup file or copy the original file from the distribution medium to the Files folder and overwrite the corrupt file. The sensors should then be recalibrated.

17. Set up your session. For each valve (by using the Valve Selection buttons on the left) select the corresponding valve chamber letter (as found on top of the HiCycle Module) which the Scan Valve is positioned, then enter the data pertaining to that valve:
    - Trial title
    - Date (automatic)
    - Trial number
    - Valve ID
    - Fluid Density
    - Fluid Temperature (as recorded on the HiCycle Controller or other calibrated thermometer)
    - HiCycle Unit number
    - Cycles counter – Enter the number of cycles displayed on the Counter of the Controller
    - Operator ID
    - Comments for reference
    - Adjust Analog Capture Variables (bottom right):
        - Drive amplitude from the reading on the *HiCycle* Controller amplitude control.

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- Chamber resistance - flow restrictor reading from the *HiCycle* Module for each valve loaded chamber.

8.2 Calibration Wizard – Navigation

The calibration process is a step by step sequence guided by the Calibration Wizard which explains each step of the process leading to measured offsets or gains for the system. The pressure calibration values are shown in the Support Information area on the right side of the HiTest screen.

While completing the Wizard, users accept or edit the current values read from the calibration file or proceed to measure new values by following the Wizard. Users can loop back to the start of the sequence to recheck values.

Close Window At any time during the calibration process, users can abort by clicking the Close button in the upper right corner of the Wizard panel. This immediately cancels calibration mode without updating the Pressure Calibration Values.

Reduce to Thumbnail As a user moves through the calibration sequence, the acquisition system continues sampling voltages in the background and shows these on the graph that is now behind the Wizard window. Users can view the graph fully by collapsing the Wizard window down to a thumbnail in the lower left corner of the HiTest window. Clicking on this thumbnail returns the Calibration Wizard window to its full size to continue calibrating.

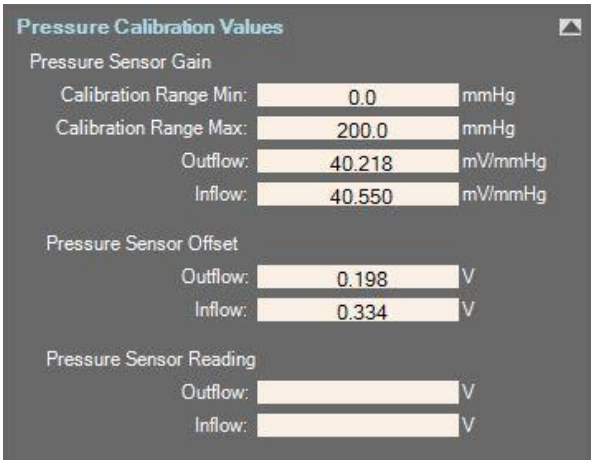
8.3 Pressure Calibration

The pressure transducers can remain installed in the Scan Valve and can be calibrated in place. The pressure transducers must be filled with fluid to be calibrated. When pressure measurements are not being made, Scan Valve should be aligned between chambers to minimize fatigue of the pressure transducers. The HiTest software guides an operator through a two point pressure calibration and static head calibration.

NOTE: Re-zeroing of the transducers and re-calibration of the pressure measuring system is required prior to accumulating data.

1. Turn the Controller on and ensure the amplitude is set to 0. Put the Controller in Standby mode.
2. Align Scan Valve with chamber connected to the Pressure Head connection.
3. Select the Calibration button from the Top Toolbar. Calibration can also be initiated through the All Menu button and then selecting Calibrate sensors.
4. Select the most recent calibration file.

5. At this stage, HiTest displays the Pressure Calibration Values table on the right hand side of the screen. This table displays the saved values entered during the process derived from the calibration file.



The Calibration Wizard will now begin. Complete the Wizard entries through the 5 steps of Pressure Calibration to edit or calculate outflow and inflow sensor gains and the 3 steps of Sensor Static Head Zeroing to edit or measure the zero offsets for these sensors. When using the ViVitro Labs Data Acquisition System, the following procedure is suggested for adjustment of the amplifiers and calibration of both pressure transducers simultaneously.

1. Step 1 of 5 of Pressure Calibration will prompt the user to either accept current calibration values or to proceed with calibration. Calibration values may also be manually entered and saved by clicking Accept.

Pressure Calibration (1 of 5)
Operators have the option of using the calibration wizard or entering outflow and inflow calibration values manually and jumping directly to Zero calibration:
Current calibration gain factors:
Outflow Sensor (Ch 1): 0.000 mV/mmHg
Inflow Sensor (Ch 2): 0.000 mV/mmHg
Click ACCEPT to use the edited values or PROCEED to start the calibration wizard.
Select:
Step Back
Accept
Proceed

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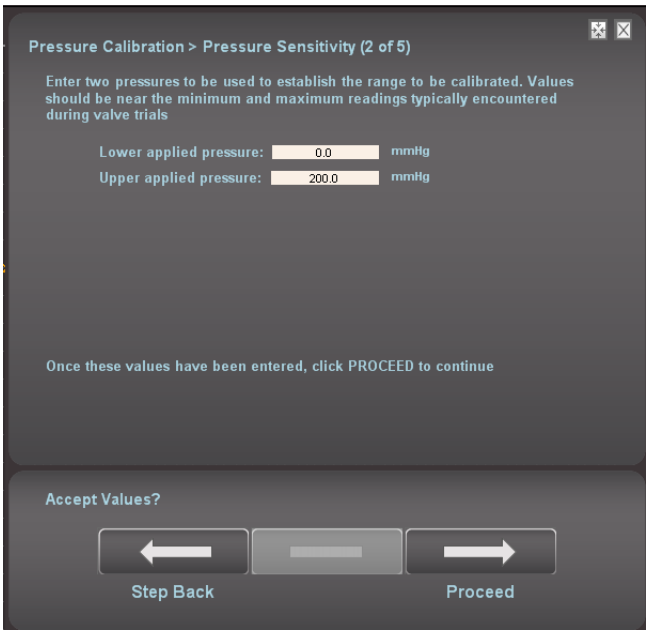
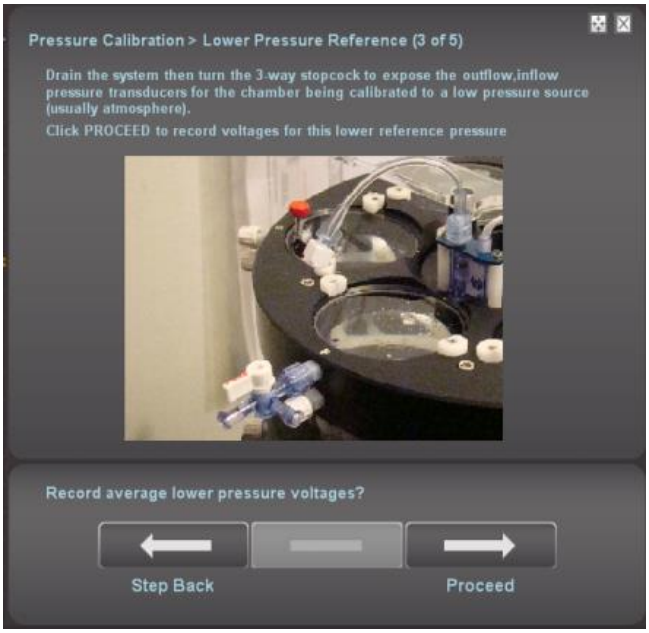
2. On step 2 of 5, the program prompts for values of lower and upper calibration pressures. They should be chosen to span the expected working range of the transducers e.g. 0, 200 mmHg.


Pressure Calibration > Pressure Sensitivity (2 of 5)
Enter two pressures to be used to establish the range to be calibrated. Values should be near the minimum and maximum readings typically encountered during valve trials
Lower applied pressure: 0.0 mmHg
Upper applied pressure: 200.0 mmHg
Once these values have been entered, click PROCEED to continue
Accept Values?
Step Back
Proceed

3. Step 3 of 5 requires the user to drain the system to capture the lower pressure reading specified in Step 2 of 5. Refer to section 6.8 for instructions on draining the chambers. When recording the average lower pressure voltage, ensure the lid is removed from the chamber or a stopcock on the chamber is open to atmosphere if 0 mmHg was the selected lower pressure reference specified in Step 2 of 5.


Pressure Calibration > Lower Pressure Reference (3 of 5)
Drain the system then turn the 3-way stopcock to expose the outflow,inflow pressure transducers for the chamber being calibrated to a low pressure source (usually atmosphere).
Click PROCEED to record voltages for this lower reference pressure
Record average lower pressure voltages?
Step Back
Proceed

4. Step 4 of 5 prompts the user to pressurize the chamber to the previously entered upper pressure reference limit (typically 200 mmHg) using a manometer as a reference.

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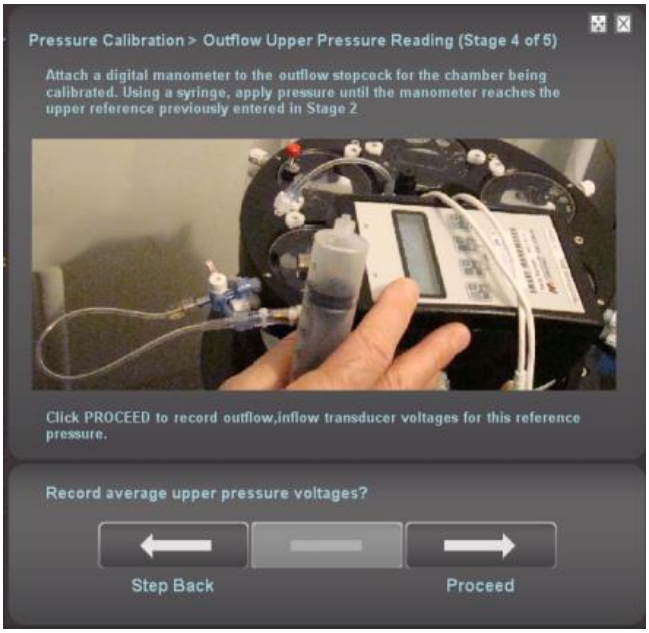
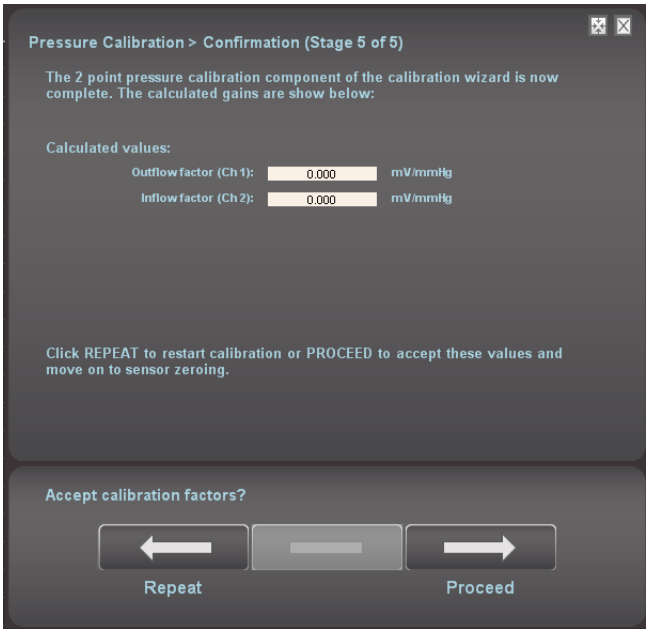

Pressure Calibration > Outflow Upper Pressure Reading (Stage 4 of 5)
Attach a digital manometer to the outflow stopcock for the chamber being calibrated. Using a syringe, apply pressure until the manometer reaches the upper reference previously entered in Stage 2
Click PROCEED to record outflow,inflow transducer voltages for this reference pressure.
Record average upper pressure voltages?
Step Back
Proceed

5. Step 5 of 5 displays the newly calculated pressure calibration values in mV/mmHg. A warning will be displayed if the gains deviate by > 1 mV/mmHg and offsets deviate by > 15 mV from previous calibration values.

   NOTE: The warning may appear if new system components have been installed. If the warning appears when components haven't been replaced, repeat the calibration. If the warning persists, consider replacing the pressure transducers. See section 9.6.

   The user can click Proceed to accept the values or click Repeat to calibrate again.


Pressure Calibration > Confirmation (Stage 5 of 5)
The 2 point pressure calibration component of the calibration wizard is now complete. The calculated gains are show below:
Calculated values:
Outflow factor (Ch 1): 0.000 mV/mmHg
Inflow factor (Ch 2): 0.000 mV/mmHg
Click REPEAT to restart calibration or PROCEED to accept these values and move on to sensor zeroing.
Accept calibration factors?
Repeat
Proceed

The Calibration Wizard will then proceed to the Sensor Static Head Zeroing portion of the calibration.

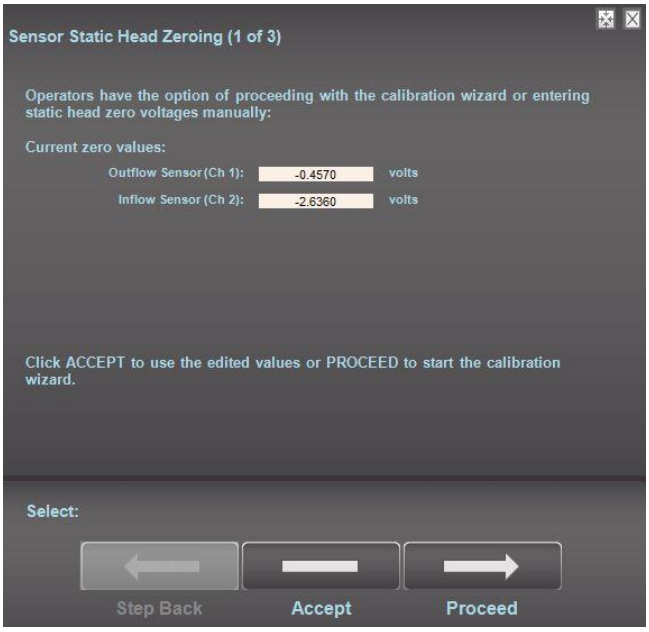
8.4 Sensor Static Head Zeroing

Zeroing captures the static head pressure difference between the outflow and inflow sensors caused by their different vertical locations. The chamber's fluid column that sits above the sensors is first opened to the atmosphere with the system static and then outflow/inflow sensor readings taken.

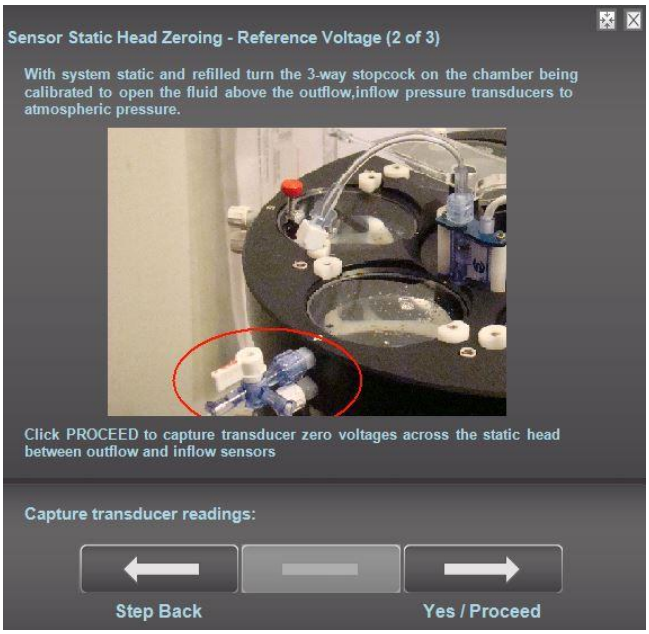
Re-establishing static head zero offset measurement is the most common of the calibration processes and best practice requires that this be done frequently within a session, particularly before starting a new valve data accumulation.

Follow the Calibration Wizard to complete steps 1 through 3.

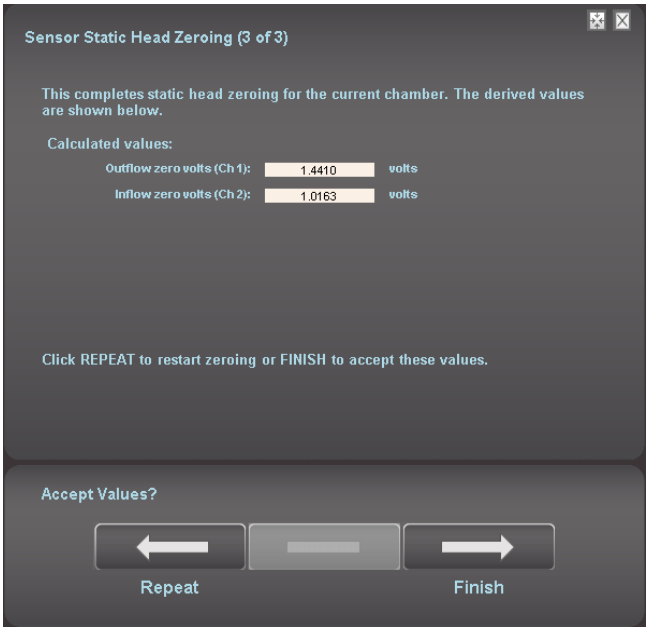
1. Step 1 of 3 of the Sensor Static Head Zeroing will prompt the user to either accept current calibration values or to proceed with calibration.


Sensor Static Head Zeroing (1 of 3)
Operators have the option of proceeding with the calibration wizard or entering static head zero voltages manually:
Current zero values:
Outflow Sensor (Ch 1):
-0.4570
volts
Inflow Sensor (Ch 2):
-2.6360
volts
Click ACCEPT to use the edited values or PROCEED to start the calibration wizard.
Select:
Step Back
Accept
Proceed

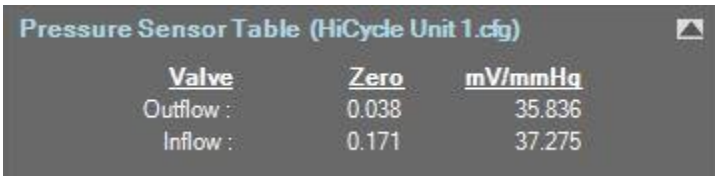
2. Step 2 of 3 requires the user to fill the system. See section 6.5 for instructions on filling the system.


Sensor Static Head Zeroing - Reference Voltage (2 of 3)
With system static and refilled turn the 3-way stopcock on the chamber being calibrated to open the fluid above the outflow,inflow pressure transducers to atmospheric pressure.
Click PROCEED to capture transducer zero voltages across the static head between outflow and inflow sensors
Capture transducer readings:
Step Back
Yes / Proceed

3. Step 3 of 3 displays the newly calculated static head calibration values in Volts. The user can click Finish to accept the values or click Repeat to calibrate again. If Finish is clicked, the user is given the option of saving the calibration file (.cfg) as a new file name.


Sensor Static Head Zeroing (3 of 3)
This completes static head zeroing for the current chamber. The derived values are shown below.
Calculated values:
Outflow zero volts (Ch 1): 1.4410 volts
Inflow zero volts (Ch 2): 1.0163 volts
Click REPEAT to restart zeroing or FINISH to accept these values.
Accept Values?
Repeat
Finish

Once the Calibration Process is completed, the Pressure Sensor Table displays the values within the Support Information Area on the right hand side of the screen.



NOTE: If calibration values begin deviating from their historical ranges, the pressure transducers should be replaced. During calibration a warning will be displayed if the gains deviate by > 1 mV/mmHg and offsets deviate by > 15 mV from previous calibration values. See section 9.6 for instructions on pressure transducer replacement. Contact ViVitro Labs to obtain replacement parts (Pressure Transducer part number 06146).

NOTE: Risk analysis requires that this calibration file be protected by a checksum and, should the file be accidentally corrupted, users are notified and given the opportunity to stop the program and recover the original calibration file from the distribution medium.

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8.5 Module Tuning

Since all test samples are all driven simultaneously in a HiCycle, changing the load on one valve may affect other valves. Therefore, system adjustment is an iterative process followed until the desired operation of all valves is achieved and the system is “tuned”.

NOTE: For best tuning, it is advised to operate the system with valves of the same size and type in each chamber. It may be possible to run with dissimilar samples. This can only be determined by experiment.

NOTE: Adjusting the window retention clamps after tuning the system will alter the operating pressures in the chamber. Ensure clamps are secure prior to tuning.

The following sequence for tuning is suggested:

1. Set all Module restrictors to 50% (if installed valves are all the same size, otherwise set lower restrictor values for small sizes and higher restrictor values for large sizes).
2. Monitor a chamber and increase drive amplitude until transvalve pressure reaches target.
3. Scan remaining chambers with Scan Valve and adjust restrictors to achieve target pressures.
4. Monitor valve opening with stroboscope.
5. If opening is excessive, reduce amplitude, scan chambers and re-adjust restrictors for target pressure.
6. If opening is insufficient, increase amplitude, scan chambers, re-adjust restrictors for target pressure.

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8.6 Data Accumulation Procedure

Before data accumulation, operators must adjust the HiCycle Controller until the test parameters satisfy the intended test criteria. The system must also be tuned as described in section 8.5 and calibrated as described in section 8.3 prior to data accumulation. Periodically over the life of the trial, users will need to accumulate cycle measurements, e.g. every 10,000,000 cycles. This process would be carried out for each valve mounted in the HiCycle Module.

A running average is kept of 3 critical values during the Accumulation Process:

- Peak Differential Pressure (mean and standard deviation)
- Percent of each cycle that meets or exceeds the target differential pressure (mean and standard deviation) (5% rule)
- Percent of cycles exceeding the 5% rule (95% rule)

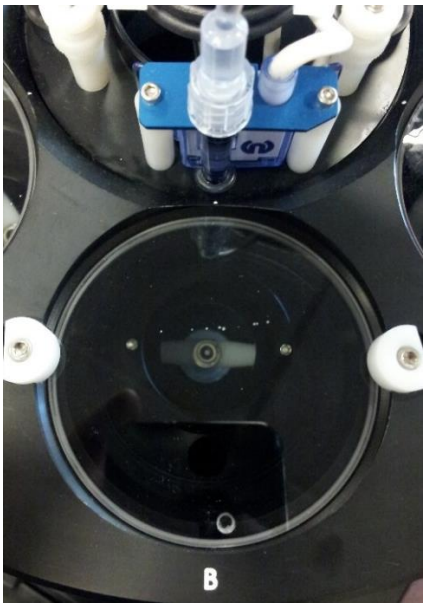
The document ISO 5840-3:2013 in Annex O.7 i) Report requirements states that a durability assessment report should include:

> *"verification that targeted pressures across the closed valve were attained for at least 5 % of each cycle during at least 95 % of the test cycles"*

NOTE: System must be calibrated and tuned to meet the test criteria in live running mode prior to data accumulation.

NOTE: Prior to accumulating large amounts of data, ensure there is adequate storage space on the computer hard drive to prevent loss of data.

1. Rotate the HiCycle Module Scan Valve to the desired chamber.



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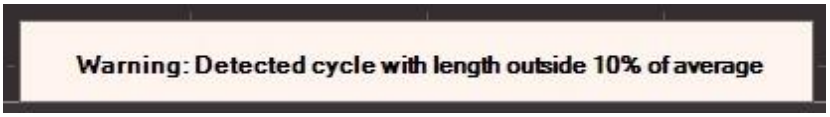
2. Select the corresponding valve letter on the software.

3. Verify that all information entered in the Session Information area is correct.
4. If the warning below is displayed during Live Running, increase the Sampling Rate in the A-D Acquisition Control area and click Synchronize HiCycle.



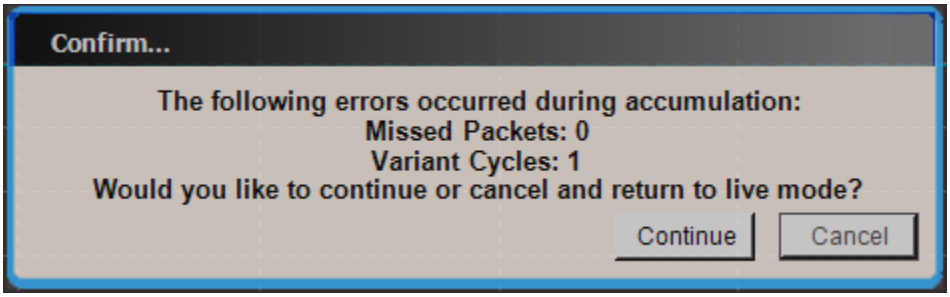
5. Click the Accumulate button

to begin the accumulating process;
6. A new window will appear asking the user for the number of cycles to be accumulated. The number of cycles to be accumulated must be between 100 and 100,000. Click OK. Progress of the accumulation is indicated at the bottom of the Process Display area.

   NOTE: Should the user wish to abort the accumulation. Click the Stop Accumulate button.

Click No to discard the data accumulated.

7. If the warning shown below is displayed at the end of the accumulation cycles, increase the Sampling Rate in the A-D Acquisition Control area and click Synchronize HiCycle. The accumulation must then be repeated. The warning will appear each time a .TST file containing an error is opened.



8. Once the accumulating is complete an information window will appear prompting the user to view static test data from the accumulation. Click continue to view and save the data.
9. While viewing the data, a .csv file can be saved by clicking the .csv save button and a .html report can be saved by clicking the .html report button.
10. After reviewing the Data, click Save Data and Resume

to save the accumulated data as a .tst file and resume Live Running mode. A dialog box will appear asking the user if they wish to save the data. If yes is selected another window will open allowing the

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user to save the data collected. Alternatively, the Save button can be clicked and the Save Data and Resume button will change to Resume Live.

NOTE: The software automatically creates a file name according to entered identifiers, date and time.

11. To open a previously saved accumulation, click the open button.

    NOTE: Before opening a previously saved accumulation, any unsaved accumulated data should be saved to prevent loss of data.

    After opening a file, the user will have the opportunity to create reports or export .csv files before resuming live acquisition.

    Repeat the process for each valve mounted within the HiCycle Module.

8.7 Diagnostics

When viewing .tst files in a text viewing program such as notepad, they display two lines of diagnostic information for the accumulation (see Section 8.8 below). Warnings will also be displayed when opening the .tst file in HiTest to alert the user if either of the diagnostic criteria are detected.

```
Diagnostics
Missed scan packets during accumulation:0
Cycles with sample counts outside 10% of average:0
```

If either the missed scan packets during accumulation or cycles with sample counts outside 10% average are greater than 0, the data should be discarded. If the number of cycles with sample counts outside 10% average is greater than 0, increase the Sampling Rate during live running mode in the A-D Acquisition Control area, click Synchronize HiCycle, and accumulate data again.

8.8 TST Files

Tst files are created by HiTest during accumulation and contain the raw data used by HiTest.

Saving, Opening and Printing a .TST File

1. Using the Save button , save the current data to a .tst file. The .tst file contains data for the number of cycles in view. A save window will be displayed and enable the user to customize the file name. Alternately, the user can opt to use the automatically assigned file name created from valve identifier information, date and time.

   NOTE: the session will remain static until otherwise instructed, therefore enabling the operator to carry out data analysis as required.

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2. To retrieve the most recent .tst file use the Open button during live accumulation and select the desired .tst file (default directory C:\Program_Files\ViVitro_Labs\HiTest\Files). A new window will open explaining that the operator will be entering a static mode indicating that live data is not presently being viewed.

   NOTE: This action will enable the operator to carry out print, snapshot and excel export activities pertaining to the data saved.

3. To return to the Live Accumulation click on the Resume Live button.

4. To view the .tst file in text form, open the desired .tst file from its file location in a text editing program such as notepad.

```
#Version#,00050032

Session Information
Trial Title:Sample
Trial Date and Time (mm-dd-yyyy hh:mm):09-18-2014 16:05
Trial Number:ABC1234
HiCycle Unit ID:HC-1
Operator ID:VV

Valve Information
Valve ID:123456789
HiCycle Valve Chamber:A
Fluid Density (g/mL):0.0
Fluid Temperature (degC):40
HiCycle Chamber Resistance Restrictor Setting (%):8
HiCycle Controller Amplitude Dial Setting:1.10
HiCycle Cycles Counter (cycles to date):99

System Settings
HiCycle Drive Frequency (BPM):600
Sample Size (Number of Accumulated Cycles):100
Sampling Rate (samples/sec):4000
Samples per Cycle:400
Outflow Pressure Transducer Gain (mV/mmHg):40.000
Outflow Pressure Transducer Offset (V):0.190
Inflow Pressure Transducer Gain (mV/mmHg):40.000
Inflow Pressure Transducer Offset (V):0.324

Comments:Sample data

Results
Target Peak Differential Pressure Across Closed Valve (mmHg):-100
Mean Peak Differential Pressure Across Closed Valve (mmHg):-164.2
Standard Deviation of the Peak Differential Pressure Across Closed Valve (mmHg):14.8
% of Test Cycles Meeting the Target Peak Differential Pressure:100.0
Mean % of Cycles Meeting Target Peak Differential Pressure (%):8.2
Standard Deviation of the % Cycles Meeting Target Peak Differential Pressure (%):1.1

Diagnostics
Missed scan packets during accumulation:0
Cycles with sample counts outside 10% of average:0


Waveform voltage data (V)
Number of Samples:40013
Outflow Pressure Transducer, Inflow Pressure Transducer, Sync
-4.6334840, -2.9415890, 5.0112920
-4.4122310, -2.9110720, 5.0131230
-3.9743040, -2.6098630, 5.0070190
-3.4906010, -2.5744630, 5.0015260
```

HiTest .tst data file

8.9 Running a Report

HiTest reports capture snapshots of the data during a valve's durability testing. Results displayed on the report enable the user to identify the trial, valve, HiCycle Unit and selected value used for the trial. A screenshot of the Process Display area is provided for visualization of the waveforms during the accumulation process.

Using the "Print Icon" a report is created within an HTML document. These files are automatically saved to your Files folder within the software directory. To print the report, follow these instructions:

Opening and Printing a Report

To keep a physical copy of the report users can:

1. Access the file via Explorer under C:\Program_Files\ViVitro_Labs\HiTest\Files.

   NOTE: The file will be identified with .html format. The file name will be the identifier information entered for the valve and the date and time.

2. Open the desired report.

   NOTE: This will open in a Browser or the computers preferred .html file viewing program.

3. Select to print from the menu.

Reports can be created after key accumulation processes have been completed and saved. The .tst files are saved after the accumulation process and by default are saved in C:/Program Files/ViVitro Labs/HiTest/Files. The .tst files can be opened to analyze previously saved data accumulations where reports can be created to demonstrate or record this data.

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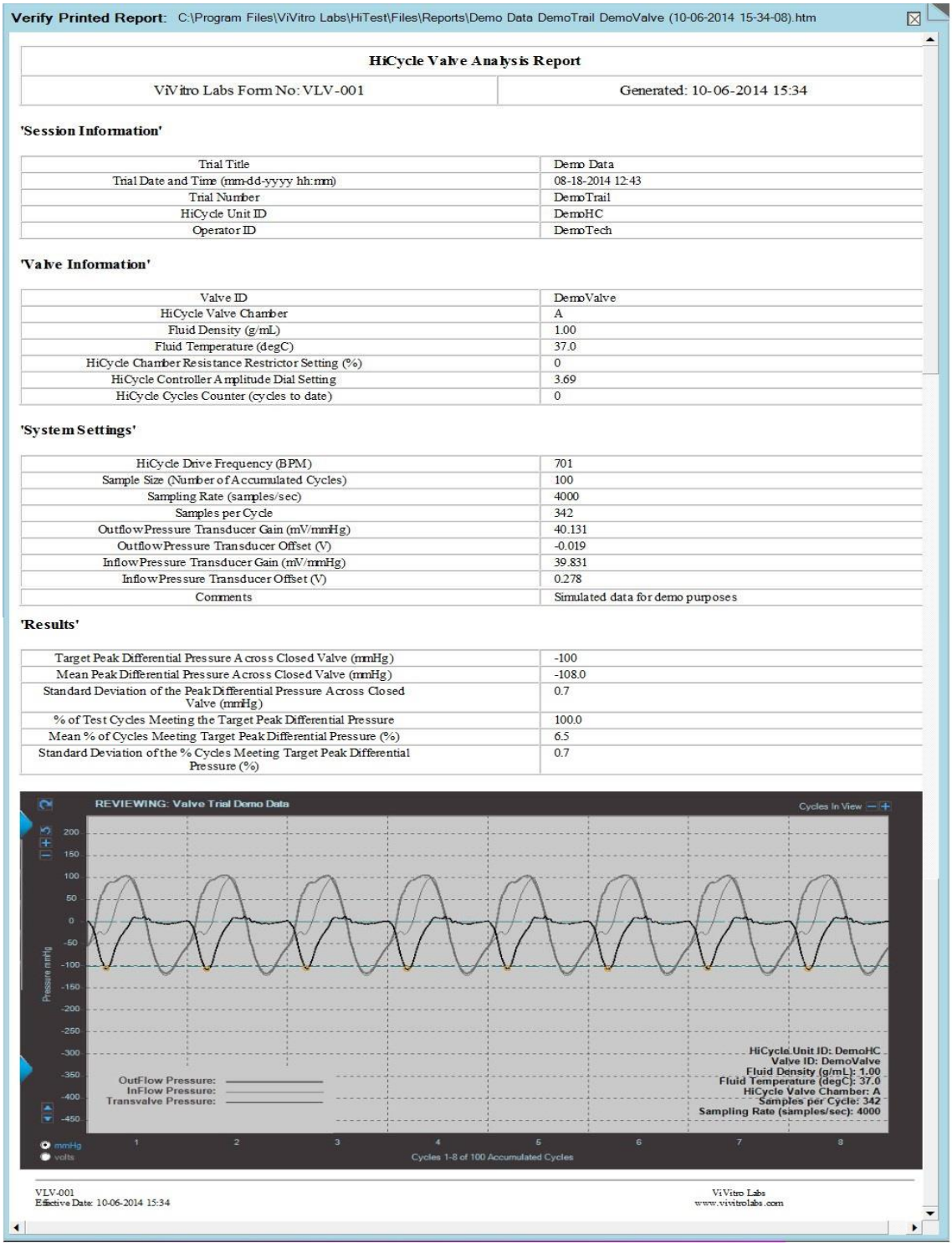

Verify Printed Report: C:\Program Files\ViVitro Labs\HiTest\Files\Reports\Demo Data DemoTrail DemoValve (10-06-2014 15-34-08).htm

HiCycle Valve Analysis Report

ViVitro Labs Form No: VLV-001	Generated: 10-06-2014 15:34

'Session Information'

Trial Title	Demo Data
Trial Date and Time (mm-dd-yyyy hh:mm)	08-18-2014 12:43
Trial Number	DemoTrail
HiCycle Unit ID	DemoHC
Operator ID	DemoTech

'Valve Information'

Valve ID	DemoValve
HiCycle Valve Chamber	A
Fluid Density (g/mL)	1.00
Fluid Temperature (degC)	37.0
HiCycle Chamber Resistance Restrictor Setting (%)	0
HiCycle Controller Amplitude Dial Setting	3.69
HiCycle Cycles Counter (cycles to date)	0

'System Settings'

HiCycle Drive Frequency (BPM)	701
Sample Size (Number of Accumulated Cycles)	100
Sampling Rate (samples/sec)	4000
Samples per Cycle	342
Outflow Pressure Transducer Gain (mV/mmHg)	40.131
Outflow Pressure Transducer Offset (V)	-0.019
Inflow Pressure Transducer Gain (mV/mmHg)	39.831
Inflow Pressure Transducer Offset (V)	0.278
Comments	Simulated data for demo purposes

'Results'

Target Peak Differential Pressure Across Closed Valve (mmHg)	-100
Mean Peak Differential Pressure Across Closed Valve (mmHg)	-108.0
Standard Deviation of the Peak Differential Pressure Across Closed Valve (mmHg)	0.7
% of Test Cycles Meeting the Target Peak Differential Pressure	100.0
Mean % of Cycles Meeting Target Peak Differential Pressure (%)	6.5
Standard Deviation of the % Cycles Meeting Target Peak Differential Pressure (%)	0.7

VLV-001
Effective Date: 10-06-2014 15:34

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The HiTest report (shown above) provides a signoff section so that operators/supervisors can verify the status of the data collected.

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8.10 Taking a Snapshot

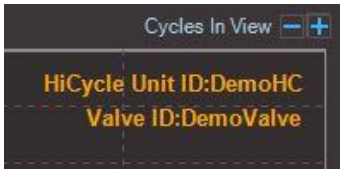
Snapshots enable the user to display represented graphs of the trial's valve cycle activity waveforms at any moment during the durability testing.

Click the Snapshot Icon to produce a .jpg file of the Process Display area. Extra data is included in the file in the bottom right corner of the graph to identify trial, valve, *HiCycle* Unit and other related data.

Customizing a Snapshot

The Process Display area can be manipulated to zoom in or out using the zoom buttons.

The focus and extent of data can be manipulated via the Cycles in View buttons in the top right corner of the screen. A minimum of 3 and a maximum of 8 cycles can be displayed.



The vertical shift of the graph can be adjusted using the arrow buttons in the lower left corner of the window.

NOTE: To capture the full HiTest screen, press SHIFT and click

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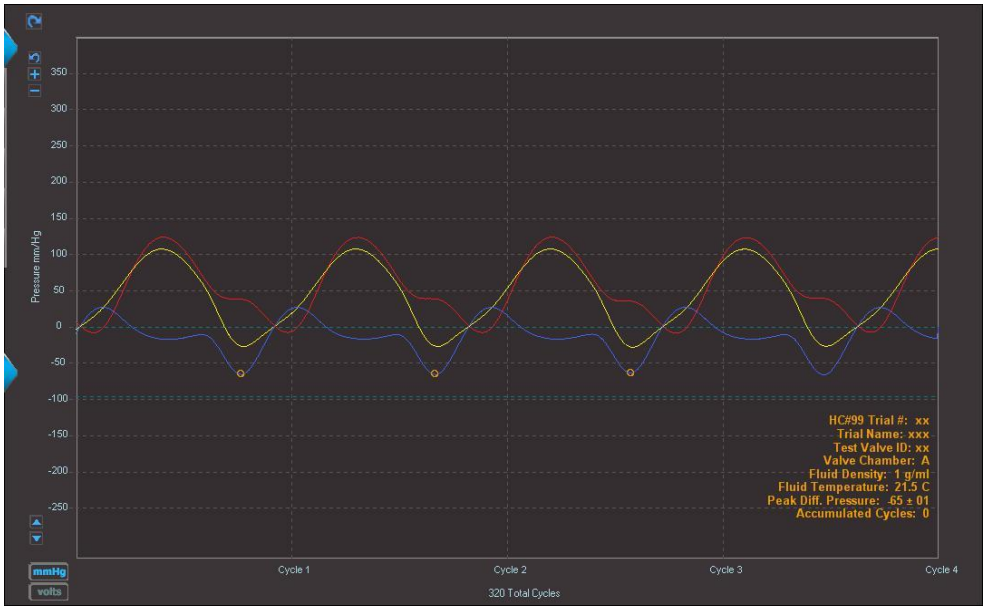

Saving, Opening and Printing a Snapshot

1. Selecting the Snapshot button will capture the Process Display area as it is seen on the screen. A save window will display to enable the user to customize the file name, alternately the user can opt to use the automatically assigned file name created from valve identifier information, data and time.

   NOTE: The Snapshot will not open immediately but be saved to the Files directory of the software for later viewing.

2. Access the file via "Explorer" under C:\Program_Files\ViVitro_Labs\HiTest\Files.

   NOTE: The file will be identified with .jpg format. The file name will be the identifier information entered for the valve and the date and time or the customized name the operator assigned.

3. Open the desired Snapshot.

   NOTE: This will open the computer's preferred .jpg file viewing program.

4. Select to print from the menu.

8.11 Exporting CSV File Data

For researchers/developers wishing to extract the data to create algorithms and calculations to provide specific results the option of producing a raw data .csv file is available. The output file contains 8 cycles of data.

By clicking on you can create a .csv file after accumulating data during the durability testing.

The output is suitable to be viewed and manipulated within a spreadsheet program. Data supplied includes trial, valve and HiCycle Unit information as well as data created during the current cycle testing and raw Inflow/Outflow pressure measurements throughout the displayed cycles.

Saving, Opening and Printing .CSV File Data

1. Selecting the .csv button after accumulating data will capture the data associated with the Process Display area as it is seen on the screen. A save window will display to enable the user to customize the file name, alternately the user can opt to use the automatically assigned file name created from valve identifier information, data and time.
2. Access the file via Explorer under C:\Program_Files\ViVitro_Labs\HiTest\Files.

   NOTE: The file will be identified with .csv format. The file name will be the identifier information entered for the valve and the date and time or the customized name the operator assigned.
3. Open the desired .csv.

   NOTE: This will open within the computers preferred .csv file viewing program.
4. Select to print from the menu.

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#Version#	50032
Session Information	
Trial Title	Test
Trial Date and Time (mm-dd-yyyy hh:mm)	09-18-2014 16:05
Trial Number	ABC1234
HiCycle Unit ID	HC-1
Operator ID	VV
Valve Information	
Valve ID	123456789
HiCycle Valve Chamber	A
Fluid Density (g/mL)	0
Fluid Temperature (degC)	40
HiCycle Chamber Resistance Restrictor Setting (%)	11
HiCycle Controller Amplitude Dial Setting	1.1
HiCycle Cycles Counter (cycles to date)	99
System Settings	
HiCycle Drive Frequency (BPM)	600
Sample Size (Number of Accumulated Cycles)	100
Sampling Rate (samples/sec)	4000
Samples per Cycle	400
Outflow Pressure Transducer Gain (mV/mmHg)	40
Outflow Pressure Transducer Offset (V)	0.19
Inflow Pressure Transducer Gain (mV/mmHg)	40
Inflow Pressure Transducer Offset (V)	0.324
Comments	User comment
Results	
Target Peak Differential Pressure Across Closed Valve (mmHg)	-100
Mean Peak Differential Pressure Across Closed Valve (mmHg)	-164.2
Standard Deviation of the Peak Differential Pressure Across Closed Valve (mmHg)	14.8
% of Test Cycles Meeting the Target Peak Differential Pressure	100
Mean % of Cycles Meeting Target Peak Differential Pressure (%)	8.2
Standard Deviation of the % Cycles Meeting Target Peak Differential Pressure (%)	1.1

Waveform pressure data (mmHg):				
Cycle #	Sample #	Outflow Pressure Transducer	Inflow Pressure Transducer	Differential Pressure
1	1	-120.587	-81.64	38.947
1	2	-115.056	-80.877	34.179
1	3	-104.108	-73.347	30.761
1	4	-92.015	-72.462	19.553
1	5	-79.541	-64.527	15.014
1	6	-67.158	-62.948	4.211
1	7	-55.394	-58.828	-3.434
1	8	-46.04	-55.41	-9.37
1	9	-37.099	-53.747	-16.648
1	10	-29.759	-47.719	-17.96
1	11	-24.205	-46.308	-22.103
1	12	-19.635	-42.646	-23.011
1	13	-16.598	-39.007	-22.408
1	14	-14.981	-38.549	-23.568
1	15	-14.485	-37.305	-22.82
1	16	-15.05	-39.533	-24.483
1	17	-15.889	-41.112	-25.223
1	18	-17.4	-44.775	-27.375
1	19	-18.628	-48.154	-29.526
1	20	-20.07	-49.375	-29.305
1	21	-21.603	-49.184	-27.581
1	22	-23.572	-49.337	-25.765
1	23	-25.944	-47.147	-21.203
1	24	-27.264	-47.819	-20.554
1	25	-29.156	-46.636	-17.48
1	26	-29.469	-46.262	-16.793
1	27	-29.233	-46.201	-16.968
1	28	-28.332	-44.82	-16.488
1	29	-26.692	-45.103	-18.41
1	30	-23.808	-40.563	-16.755
1	31	-20.81	-39.808	-18.998
1	32	-16.888	-36.573	-19.685
1	33	-12.784	-33.079	-20.295

HiTest .csv data output

8.12 Log Files

As an ongoing background feature of HiTest, log files are created each time a user instructs the software to do an activity. These files can be accessed via the C:\Program_Files\ViVitro_Labs\HiTest\Files\Logs directory as a .log file. Log files are for technical support purposes and are not intended to be used as a record for valve data.

Operators can append particular data to a .log file by selecting the Append button. This creates a raw data file of activities and data of the present moment within the durability testing cycle.

Saving, Opening and Printing a .LOG File

1. Selecting the Append button will capture the data associated with cycle's data at that specific time. A save window will display to enable the user to customize the file name.

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Alternately, the user can opt to use the automatically assigned file name created from valve identifier information, date and time.

NOTE: The .log file will not open immediately but be saved to the Files directory of the software for later viewing.

2. Access the file via “Explorer” under C:\Program_Files\ViVitro_Labs\HiTest\Logs.

NOTE: The file will be identified with .log format. The file name will be the identifier information entered for the valve and the date and time or the customized name the operator assigned.

3. Open the desired .log file.

NOTE: This will open within the computers preferred .log file viewing program.

4. Select to print from the menu.

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9. System Maintenance

9.1 Chamber Cleaning

The chambers can be cleaned by soaking with mildly soapy hot water (approximately 45 degrees Celsius) and flushing with fresh water several times to clean soap residue from the chambers.

More thorough cleaning may require Module disassembly.

NOTE: KEEP ORGANIC SOLVENTS AWAY FROM THE SYSTEM AT ALL TIMES.

NOTE: DO NOT DAMAGE TEFLON™ COATING ON THE PLATE LOCATED AT THE BOTTOM OF TEST CHAMBERS.

9.2 Shroud Removal

To access the bellows and piston rods remove the shroud around the Module.

1. Remove 9 upper and 9 lower Phillip's head screws attaching shroud to Module.
2. Loosen 2 Phillip's head screws on left of Module panel.
3. Move end of shroud away from panel.
4. Tighten 2 panel screws to maintain panel alignment.
5. Loosen 2 Phillip's head screws on right side of Module panel.
6. Slide end of shroud away from panel.
7. Remove shroud.

9.3 Controller Cleaning

To clean the Controller case, use a dry cloth. If the case cannot be cleaned by dry cloth then disconnect power and use a damp cloth to wipe the exterior of the case. Use caution and do not allow liquids to enter the controller case.

9.4 Bellows Replacement

It is recommended that bellows are replaced every 50 – 100 million cycles. Should bellows need replacement, they can be removed as follows:

1. Remove Module shroud as described in section 9.2.
2. Remove bolt securing piston using 9/64" driver and then remove piston.
3. Remove bolt and washers at base of piston rod.

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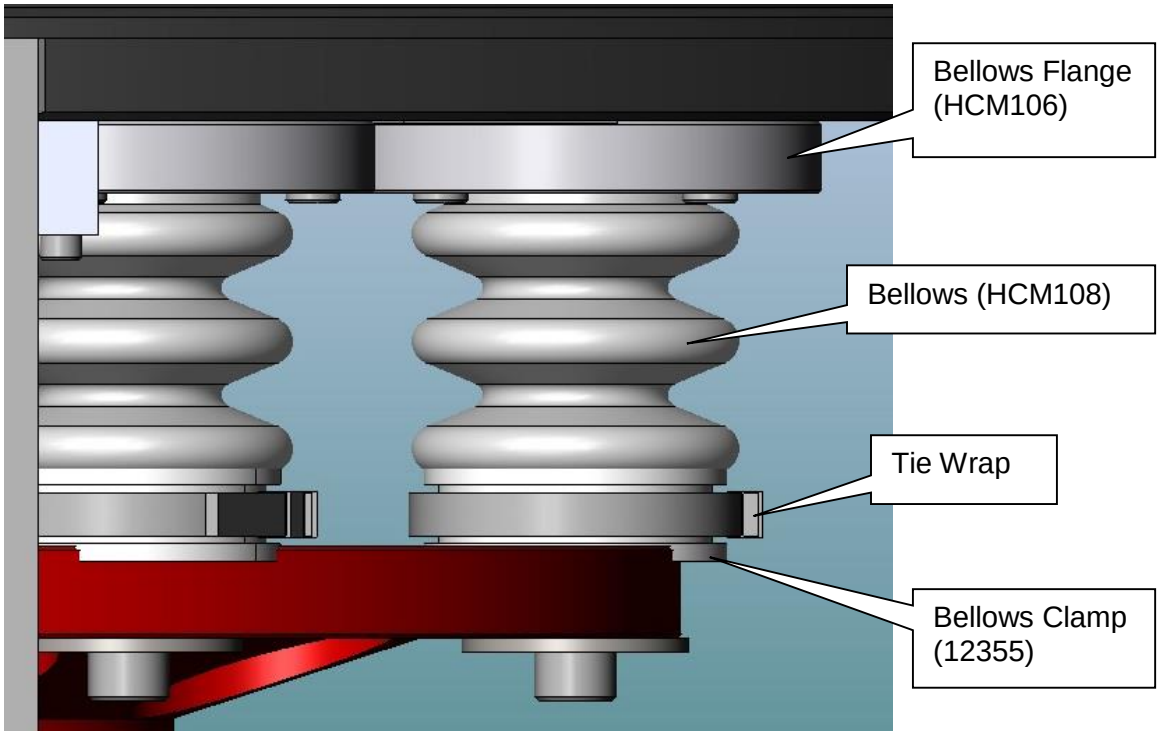
4. Remove piston rod from narrow end of Bellows
5. Remove four bolts from Bellows Flange at the wide end of bellows using 3/32” hex head key
6. Clip the existing tie wrap with side cutters



To install bellows:

1. Position clamp over wide end of bellows and secure clamp using four bolts with 3/32” hex head key to Module base plate so that bellows seals to this plate.
2. Push piston rod through neck of bellows (COUNTERBORED END OF ROD ONLY).
3. Secure piston rod to pusher plate using bolt and washers.
4. Ensure that the Bellows are not twisted or crooked.

NOTE: Misalignment of bellows can cause the bellows to rub on the piston rod. This results in premature wear on the bellows.

5. Place the two halves of the Bellows Clamp around the narrow waist of the Bellows.
6. Secure with a new 5 ¾” tie wrap.

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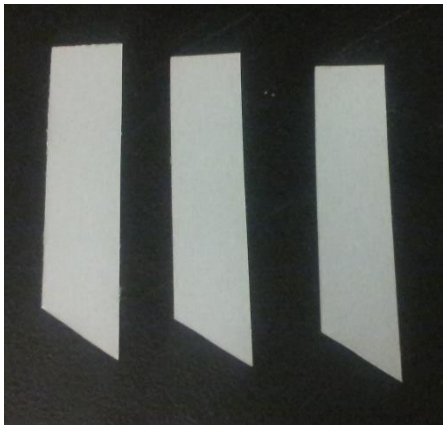
9.5 Piston Alignment

The piston rods and pistons must be centered in the cylinders for proper operation. Alignment can be checked as described in section 4.3. The pistons can be aligned as follows:

1. Remove the Module shroud as described in section 9.2 above.
2. Remove bolt in the center of all pistons using 9/64" driver and remove pistons
3. Loosen bolt at base of piston rods using 9/64" key
4. Re-insert a piston into piston cylinder and center with the three the plastic shim strips that are supplied in the accessories kit. The shims should be equally spaced by approximately 120°. The piston spoke should be perpendicular to the flow restrictor channel. The bottom surface of the piston spoke should be slightly above the top of the piston rod.



5. Move the piston rod so that the tapped hole in the top of the piston rod aligns with piston center hole.
6. Tighten bolt at base of piston rod maintaining center alignment above.
7. Insert bolt with stainless steel flat washer in central piston mounting hole and tighten.
8. Remove shim strips.
9. Check that the piston moves freely as described in Section 4.3.
10. Repeat the above steps for each piston.

9.6 Pressure Transducer Replacement

In the event that pressure transducer calibration values begin deviating from historical standard values, or erratic and noisy data is observed, the pressure transducers (part number 06146) should be replaced. When ordering new transducers from ViVitro Labs, specify that the transducer is being used in the Scan Valve (unless the alternate Pressure Head Assembly configuration shown in Section 13 is being used).

To install the Pressure Transducer in the Scan Valve:

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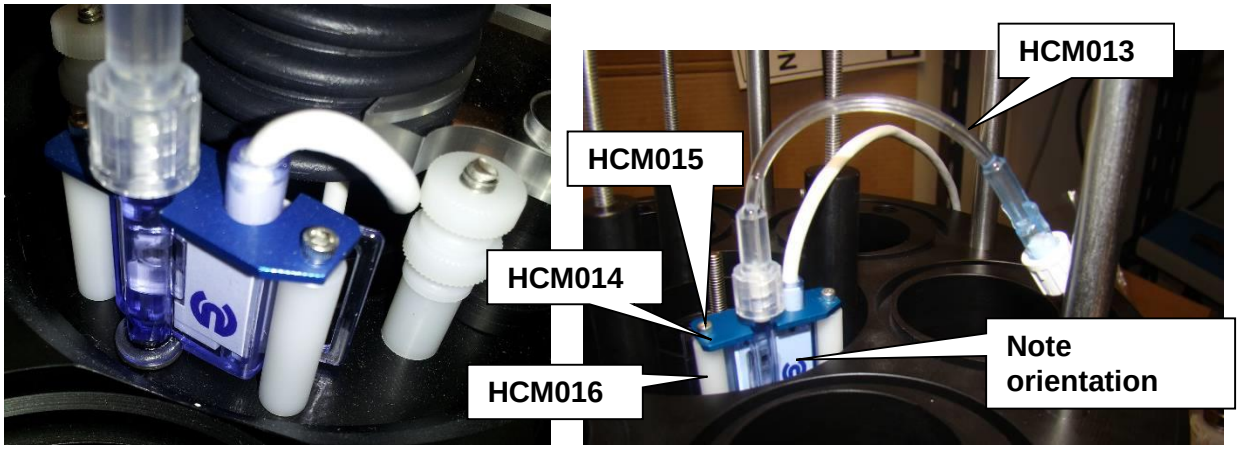
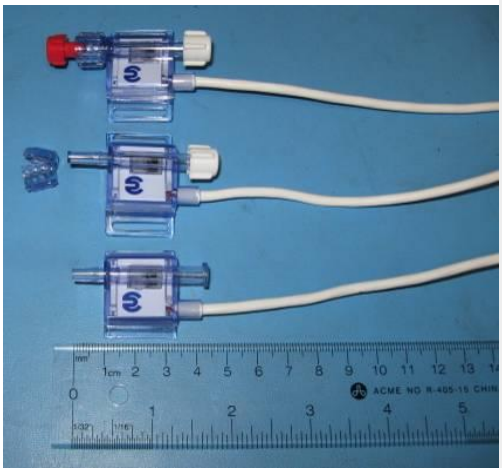
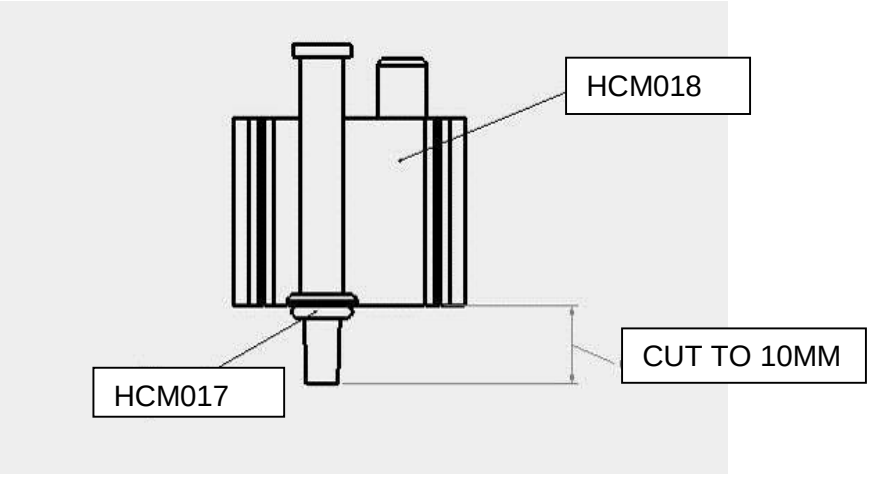
1. Insert the nipple of the Pressure Transducer (HCM018) into the Lower Scan Valve.
2. Install the Retainer Plate (HCM014) to the Pressure Transducer Standoffs (HCM016) with the 2-56 x 3/8" Modified SHCS (HCM015). Use calibrated driver to install at 2 in-lbs.
3. Tighten the Female Luer end of the Safety Cap Assembly (HCM013) onto Male Luer fitting on top of the Pressure Transducer.



If a standard transducer (typically for use with other ViVitro Labs' products) needs to be modified to be compatible with a Scan Valve, follow the steps below:

1. Break the collar from the nipple of the Pressure Transducer (HCM018). See image to the left below.
2. Cut the end of the Pressure Transducer so that the nipple is 10mm long. See image to the right below.
3. Push an O-ring (HCM017) on each of the nipple of the Pressure Transducer.





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10. HiTest 5.0 Release Notes

The following features are new in HiTest version 5.0

- Standardized output file format and trial information display
- CALIBRATING, LIVE RUNNING, and REVIEWING mode display in Top Level Toolbar
- Number of accumulated cycles displayed in REVIEWING mode
- File name and date of active calibration file displayed on Process Display
- Most recently accessed calibration file recalled during Select a Calibration File prompt
- No Sync Pulse Detected warning display in Live Running mode
- Invalid Cycle Length, Missed Data Packets, and Variant Cycles warning messages
- Warning displayed if calibration file gain deviates by > 1 mV/mmHg for gain and 15 mV for offset
- User manual download link accessible through Options menu button in Top Level Toolbar
- Backward compatible with files accumulated with previous versions of HiTest

The following are notable changes made in HiTest version 5.0:

- Calculation of the 95% and %5 rules updated to match wording in ISO 5840: 2013-3, Appendix O.7
- .RW file no longer created. All accumulated cycles are now captured in .TST file.
- CSV and TST Data Files window removed. Files are to be opened through the Open File icon on the Top Level Toolbar.
- Timestamps given to default Calibration file names
- Additional tooltip information popup windows for buttons
- Accessing File no longer displayed in title bar while selecting Calibration file

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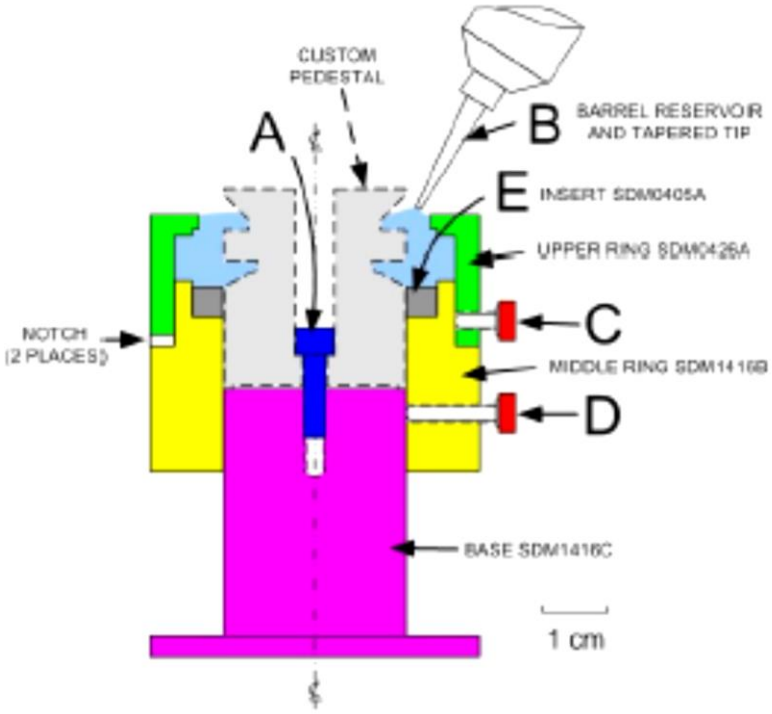
11. Appendix A - Valve Ring Molding and Mounting

Silicone rubber rings are recommended for mounting heart valves or other test specimens. The rubber rings provide a fluid seal between the device being tested and the test system flanges.



11.1 Design and Fabrication Considerations

Since each size and type of test device differs in geometry, a unique rubber ring is required in order to match and seal the appropriate boundaries on the device. The rubber ring should not interfere with the action of the device being tested. The considerations discussed here apply to the design of rubber rings for mounting mechanical or tissue valves, with or without sewing rings.

Molding jig components for valves are shown colored in the following illustration. The jig components can be separated and adjusted by loosening bolt A and thumb screws C & D.

The custom pedestal (Light GREY) is attached to the base (PURPLE) with a bolt (A). The pedestal can be the actual valve or a replica of the outer dimensions to which the rubber ring seals. It can be made of Delrin™ which has good mold release ability. When a valve or valve component is used as a mold, a thin layer of Vaseline coating on the component may facilitate mold release after vulcanization. Vaseline can be washed off using soap and water after the rubber ring cures. In general, to help minimize flow turbulence, a tapered transition should be made between the outside diameter of the rod and the inside diameter of the device inlet orifice.

For tissue valves, it is likely that the tissue will be damaged in the molding process and should therefore be cut away before molding. A tissue-less valve stent, having all component parts except issue, can facilitate molding of complex shapes. If porous fabric materials are to interface with the silicone rubber,

a thin layer of mold release (e.g. Vaseline or wax) should be applied before molding. Experimental devices, (e.g., VSI, BC bi-leaflet tissue valve) can use a plastic rather than a fabric cuff. This simplifies the design of the prototype valve and fabrication of both the mounting and rubber rings.

11.2 Molding Kit

A molding kit is supplied to assist in making rubber rings. The standard kit consists of the following parts:

- 1 each, molding jig components
- 1.8 pounds, GI-1100 Silicone rubber (Silicones, Inc.)
- 1 each, dispensing syringe (enlarged end port), 35 ml
- 0.2 pounds, GI-1100 Activator (Silicones, Inc.)
- 1 each, dispensing syringe, 1 ml
- 1 each, standard dispensing syringe, 35 ml
- 1 each, gage tapered tip, #5116TT-B (EFD Inc.)
- 100 ml, waxed paper mixing cup and wooden spatula
- 1 ml, mold release grease (Vaseline) in syringe

Optional accessories recommended for molding silicone rubber are given below. These items may be obtained from the indicated suppliers.

- 35 ml/stroke, hand-operated vacuum pump, capable of generating up to 25 inches of mercury vacuum, # P-79301-20 (Cole-Parmer Inc.)
- 365 ml, Plexiglas vacuum chamber, ¼ inch I.D. tubing, VC9991
- Air-powered dispenser Controller, gas pressure input 80-100 PSIG; gas pressure output 0-100 PSIG, #800V (EFD Inc.)
- 30 ml Barrel reservoir/pistons, #5112CP-B (EFD Inc.)

NOTE: A standard, hand operated, 35 ml syringe may also be used rather than an air powered dispenser and barrel reservoir/piston combination, however, considerable force is necessary to inject the rubber mixture. The tapered tip (e.g. #5116TT-B) should be attached to the syringe before dispensing the rubber.

11.3 GI-1100 Silicone Rubber Properties

This rubber is made by Silicones, Inc., P.O. Box 363, 211 Woodbine Street, High Point, NC 27260, USA. Ph (336) 886-5018, Fax: (336) 886-7122 (http://www.silicones-inc.com/). It has extremely high tear strength and is a, highly elastic, two-component, tin catalyzed RTV (Room Temperature Vulcanizing) silicone rubber.

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11.4 Mounting Ring Mold Assembly

1. The Insert **E (Dark GREY)** should be removed when making rubber rings for use in the HiCycle system.
2. With the middle ring **(YELLOW)** over the base **(PURPLE)**, attach the Custom Pedestal or device replica **(light GREY)** so that it is centered and square to the base rod **(PURPLE)**. The method of attachment will depend on the device.
3. Fill the assembly with silicone rubber (GI-1100 recommended) which has been de-aerated as described above. Prior to complete jig assembly and filling with rubber, it is recommended that rubber first be applied to deep undercut regions using the barrel reservoir and tapered tip (B). This avoids entrapping air in those regions where the flow of the viscous rubber can be problematic.
4. Place the upper ring (**GREEN**) over the middle ring **(YELLOW)** and lock with thumbscrew (C). Adjust the locked assembly (**GREEN** + **YELLOW**) on the base **(PURPLE)** so that when rubber fills the cavity, the top level of rubber is even with the top edge of the upper ring **(GREEN)** and about 2-3 mm of rubber covers the top ridge of the pedestal or device **(TAN)**. This top covering layer of rubber should be sufficient to secure the test device during forward flow conditions.

11.5 Mixing RTV Silicone Rubber

For making one rubber ring, about 15 ml volume of GI-1100 RTV silicone base is required. This volume is best measured and dispensed from the silicone base container using a modified 35 ml syringe which has a large hole in the syringe end. The silicone base is to be mixed with activator at a 10:1 ratio.

11.6 De-Aerating and Dispensing Mixture

Immediately after mixing, de-aeration of the mixed rubber to remove entrapped air is recommended. Removing air bubbles helps ensure the rubber rings sealing ability and provides uniformly dense rings. Proceed as follows:

1. Place the mixing cup with the mixed silicone rubber into a small volume vacuum chamber (e.g. part # VC9991, 365 ml chamber volume).
2. Attach a hand operated vacuum pump to the vacuum chamber using a ¼ inch I.D. hose (e.g. # P 79301-20).
3. Evacuate to about 24 inHg (610 mmHg) vacuum. The mixture will expand towards the top edge of the cup and then begin to collapse as the entrapped bubbles burst in the low pressure. The mixture should eventually collapse back to its original volume. The bursting of the bubbles can take several minutes however this time can be reduced by providing multiple evacuation/release cycles during the de-aeration process. During this cycling process, the 24 inHg (610 mmHg) vacuum condition should be maintained for about 2 minutes. Using this method, the total de-aeration time should be about 10 minutes.

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4. Release vacuum and remove the cup from the chamber
5. Pour and spatula the mixture into a 30 ml barrel (e.g. #5112CP-B) and insert piston.
6. Expel barrel air as much as possible.
7. Attach tapered tip (e.g. #5116TT-B) to loaded barrel.
8. Attach the barrel adapter assembly to an air pressure source (e.g. #800V) and set pressure to 40 psig (2069 mmHg).
9. Slowly inject the mixed silicone rubber into the cavity being careful to avoid the formation of air bubbles or entrapment of air. Allow the rubber to flow around the cavity. The time to fill the cavity with rubber should be about 10 minutes.
10. Allow the rubber to cure (vulcanize) for 16 to 18 hours at 25 C. Lower temperatures and/or low humidity will cause the cure-time to lengthen. Conversely, higher temperatures and/or high humidity will cause the cure-time to shorten.

11.7 Mounting Ring Removal

1. When the rubber is cured, dismantle the molding jig and remove the rubber ring. Remove the upper ring **(GREEN)** from the middle ring **(YELLOW)**. Two notches on the upper ring **(GREEN)** allow insertion of a blade type screw driver which can help pry the two rings apart.
2. Push the middle ring **(YELLOW)** down over the base **(PURPLE)**.
3. Peel and stretch the rubber ring off the Custom Pedestal or device **(light GREY)**.
4. The rubber ring should be trimmed of any excess rubber using small scissors. Trimming should be done carefully to prevent compromising the seal of the ring.

The device can now be mounted in the silicone ring and installed in the HiCycle Module.

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12. Appendix B - Alternate Compensation Spring Configuration

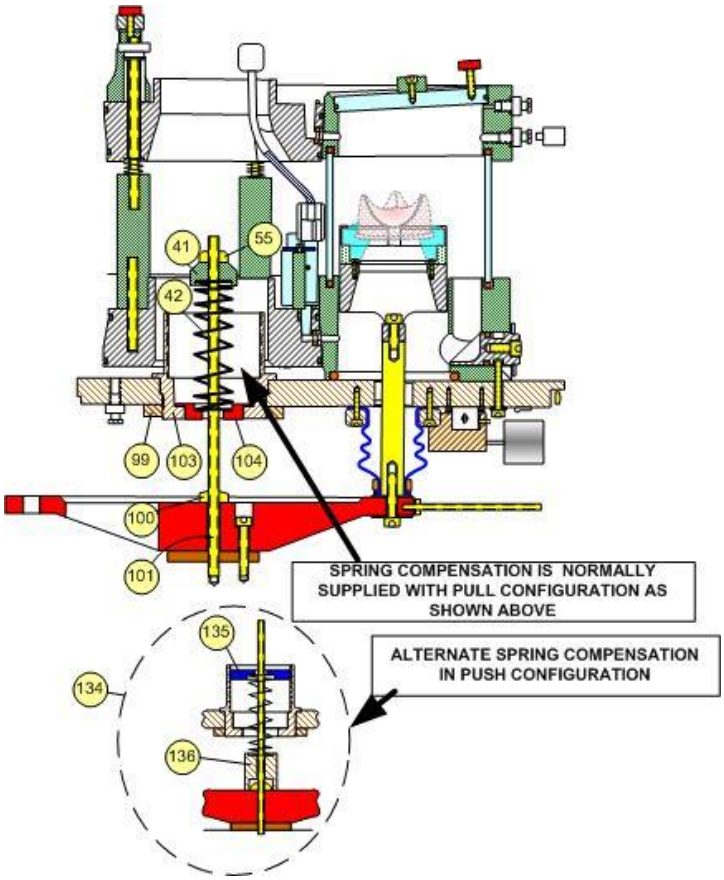
The Hi-Cycle MODULE is normally shipped with the compensating spring in PULL configuration. This is recommended for valves mounted with outflow uppermost which is preferred for visualization of valve outflow dynamics.

In this PULL configuration, the spring pulls upward on the pusher plate and counteracts the biased downward load on the plate due to the mean closed valve load being more than the load when the valve is open.

The tension in the compensating spring should be adjusted by turning the nut with the 3/8 inch driver provided so that the mean plate position under dynamic conditions, as indicated by the monitor pointer, is the same as the static no load plate position. In this way, the shaker operates efficiently and “creep” of its driver coil suspension mechanism is minimized.

It may be preferable to mount mechanical valves with inflow uppermost so that gravity assists opening. In this case, the spring configuration should be changed to PUSH as depicted by:

1. Removing nut using 3/8” driver.
2. Removing spring collar, spring & spring washer.
3. Inserting new spring spacer, spring collar and spring adjuster.



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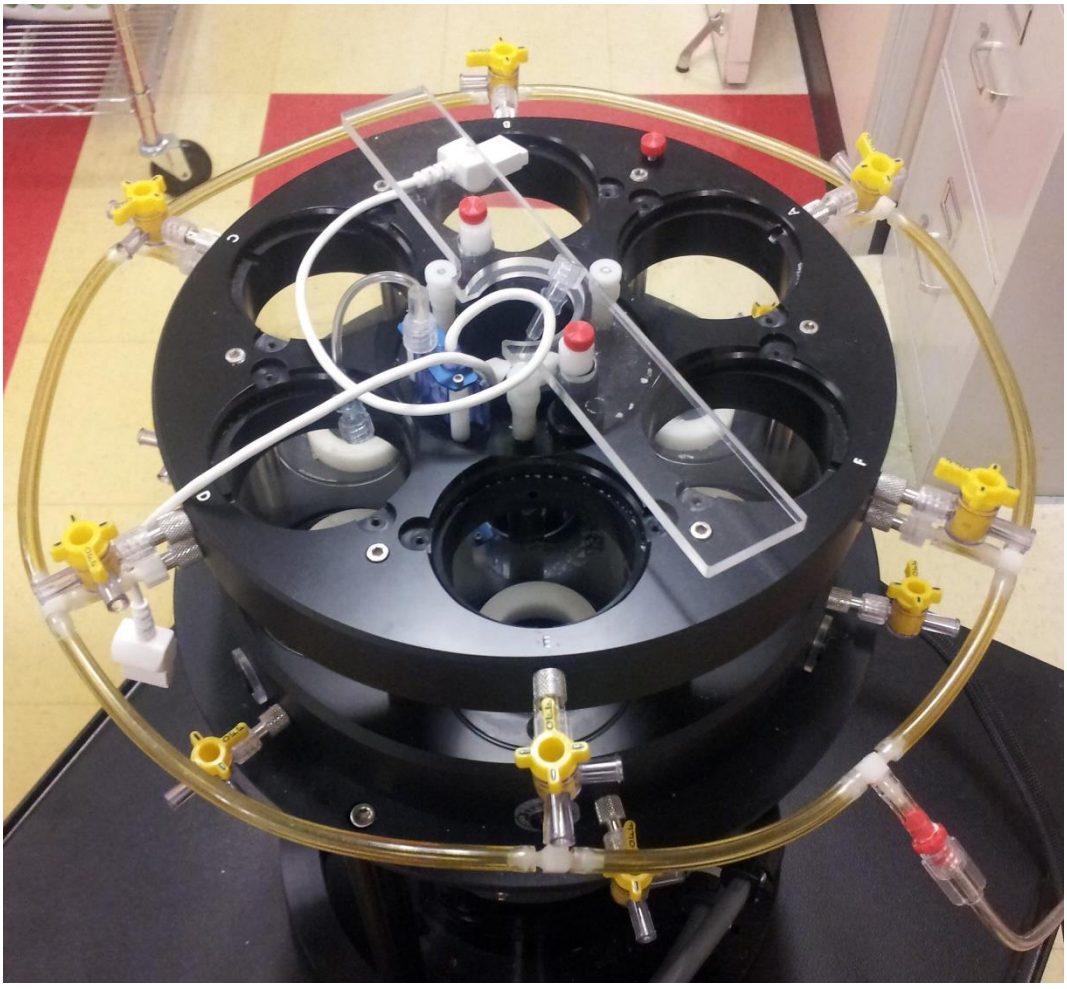
13. Appendix C - Alternate Pressure Head Configuration

Some users may wish to use connect the Pressure Head assembly and the pressure transducers directly to the external luer fittings of the HiCycle for collecting data. This configuration can easily be assembled using standard tubing and fittings.

To externally connect the Pressure Head assembly and allow for externally mounted pressure transducers, set up the HiCycle module as shown below.



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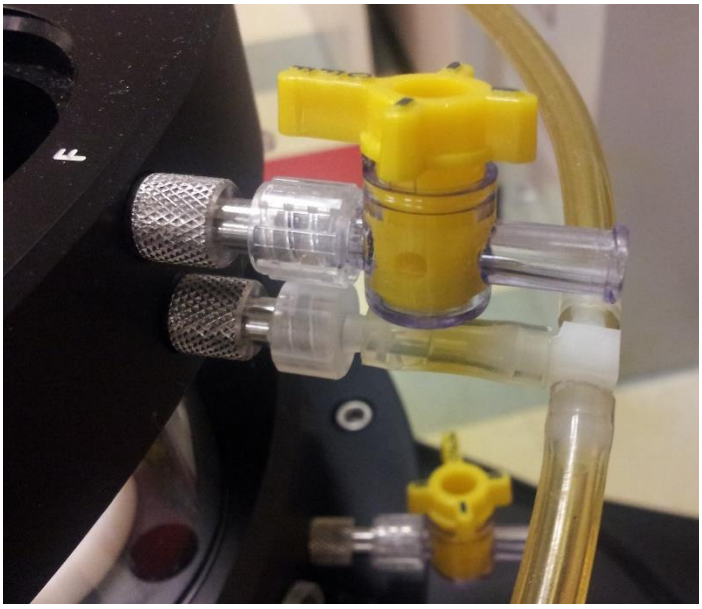

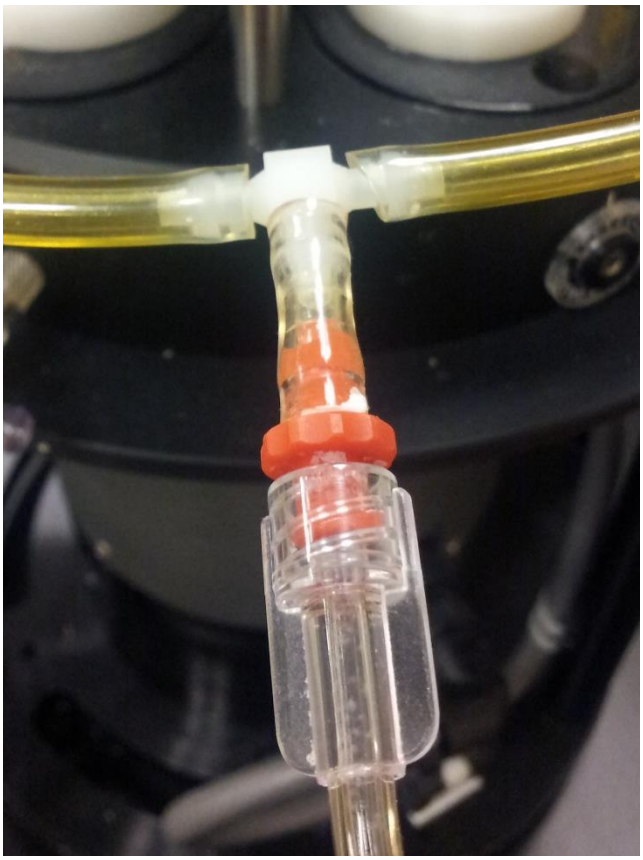

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14. Appendix D - Recommended Spare Parts Kit

The recommended spare parts for the HiCycle system are listed below. It is recommended that the parts listed be kept on hand to reduce down time in the event of a component failure. Contact ViVitro Labs to purchase a Spare Parts Kit.

Description	Part Number	Quantity
CHAMBER WINDOW	HCM046	6
HiCycle Pressure Transducer Assembly	06146	2
U-CUP TEFLON SEAL 3.441"	HCM031	2
U-CUP TEFLON SEAL 3.286"	HCM032	2
TEFLON O-RING MOD.	HCM054	2
O-RING V-036-70	HCM045	6
O-RING V-230-70	HCM060	12
O-RING V-225-70	HCM105	6
O-RING V-009-70	HCM021	18
O-RING V-110-70	HCM075	6
Tubing w/ Luer-Lock ends, 48"L	05842	1
BELLOWS	HCM108	12

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15. Appendix E - Durability Testing Step By Step Guide

15.1 Scope

This guide is intended to serve as a reference for testing once the user has already become familiar with the system through training and reading the user manual. This test method describes the testing to be performed on cardiovascular implants or cardiac valve prostheses for evaluating durability. The method may vary for different samples or test plans.

15.2 Definitions

Symbol	Definition
BPM	Frequency of operation expressed in terms of beats per minute.
Deployed valve diameter	Outer diameter (mm) of the implantable device when deployed within the target implant site in an idealized circular configuration. This measure is the size used to determine minimum device performance requirements for transcatheter valves.
AWT	Accelerated wear testing, wear testing beyond physiologic conditions e.g. beat rates >200 bpm).
Control valve	A valve with known performance, used as a benchmark during wear testing.
Regulatory test	A test conducted with the intent of submission to a regulatory body (i.e. FDA, CE). The test should fully comply with all applicable regulations.
Tuning the system	A series of fine adjustments to the accelerated wear testing system elements. This is necessary to ensure accuracy and repeatability of the test conditions from run to run.
Tissue Annulus Diameter	(TAD) Diameter in millimeters of the smallest flow area within the patient's valve annulus. This measure is the size used to determine minimum device performance requirements for surgical and mechanical valves.
Transvalvular Regurgitant Fraction	Is defined as the volume of fluid that flows through a valve in the reverse direction during one cycle and is the sum of the closing and leakage volume expressed as a percentage of the stroke volume. This assessment is conducted with sealing applied to the valve to prevent paravalvular leakage.

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Total Regurgitant Fraction	Is defined as the volume of fluid that flows through and around a valve in the reverse direction during one cycle and is the sum of the closing and leakage volume expressed as a percentage of the stroke volume. This assessment includes both transvalvular and paravalvular leakage.
Nominal Conditions	Test parameters used for acceptance criteria listed in ISO 5840. Consisting of 5 LPM at a beat rate of 70 BPM and stroke volume of 75 mL/stroke with a 35% systolic duration.
5% and 95% rule	Targeted pressures across the closed valve were attained for at least 5% of each cycle during at least 95% of all test cycles.

15.3 Summary of Tests (ISO 5840-3 Annex O)

During durability testing devices will be cycled in a durability tester at an accelerated rate. At the same time, a control device may be cycled in order to compare the performance of the test valves to that of a similar valve that is currently available in the market and that is clinically approved. Performance will be evaluated through hydrodynamic and visual inspections performed throughout the test.

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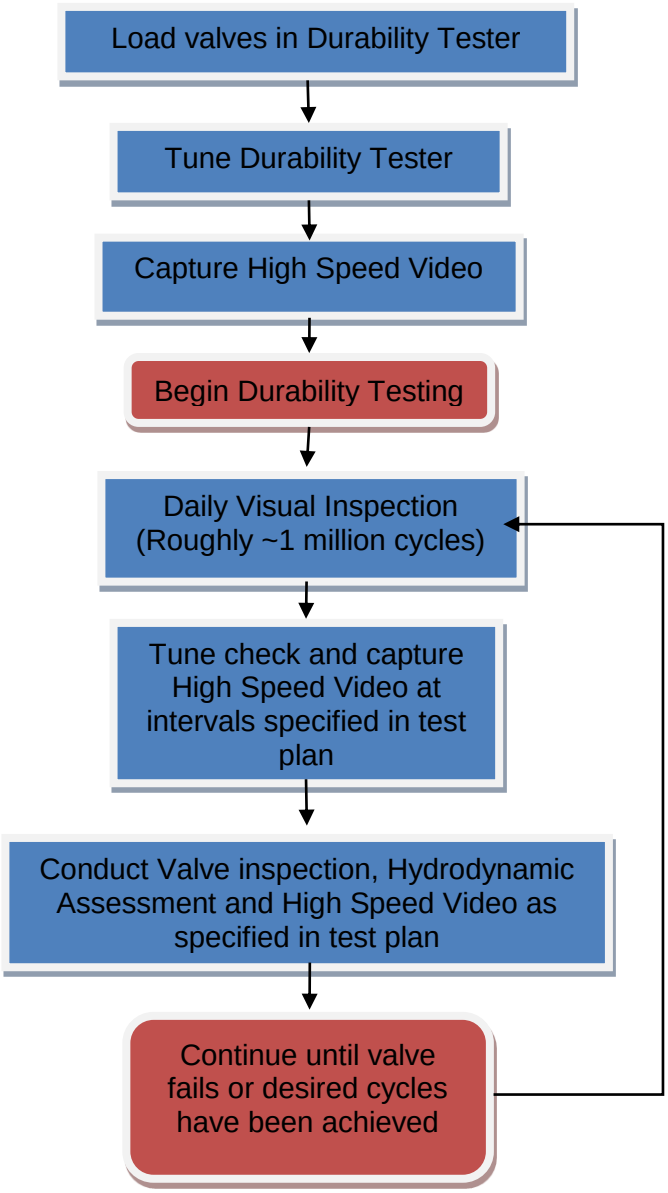
Load valves in Durability Tester
Tune Durability Tester
Capture High Speed Video
Begin Durability Testing
Daily Visual Inspection (Roughly ~1 million cycles)
Tune check and capture High Speed Video at intervals specified in test plan
Conduct Valve inspection, Hydrodynamic Assessment and High Speed Video as specified in test plan
Continue until valve fails or desired cycles have been achieved

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15.4 Equipment Required

Calibrated: Ensure equipment has a valid calibration before using

- Manometer
- Thermometer

Other:

- ViVitro Labs HiCycle
- Stroboscope
- Data Acquisition System including HiTest
- High speed video recording system
- Test fluid
- Syringe
- Laboratory notebook – for submission testing a dedicated notebook is required
- Flash light
- Hex Keys

15.5 CAUTIONS

- Always perform amplitude and restrictor adjustments slowly. Where possible ensure valves are connected to pressure transducers and showing live readings in HiTest to ensure valves are not experiencing undue stress.
- Use caution to avoid spilling test fluids as electronics reside below the module.

15.6 System Setup

1. Set up the durability tester as per section 4.
2. Turn on durability tester controller and AmPack, to allow electronics to come up to operating temperature.
3. If required turn on the heater to allow it to come up to required temperature.

15.7 Calibration

Calibration is only required for the pressure transducers. This consists of a two point pressure calibration and static head compensation. Use a manometer for pressure reference to ensure that calibration is valid.

1. Start HiTest software and open the calibration file that the software defaults to.
2. Click to enter the calibration wizard and follow the steps. For more clarification see section 0.
3. At the completion of calibration the inflow, outflow, and transvalve differential pressure should all read zero when the pressure transducers are open to the test fluid.

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4. Save calibration file.

15.8 Baseline Assessment

1. If required by the test plan conduct an initial inspection of the valves.
2. If required by the test plan conduct an initial hydrodynamic assessment.
3. Perform high speed videography to record the opening and closing characteristics of the valve in the pulse duplicator.
4. After the completion ensure all data is backed up.

15.9 Setup and Loading

Refer to section 4 for detailed instructions.

1. Set cycling frequency in accordance to test plan.
2. Reset cycle count on tester if required.
3. Fill with enough test fluid to cover the samples, but not cause overflow while installing samples.
4. Turn on heater, Set temperature set point according to test plan. Using calibrated thermometer ensure displayed controller temperature is accurate.
5. Install samples and install lids as per test plan and/or sections 6.2 and 6.3. Record location of valves in the lab notebook. If less than 6 valves are being tested, ensure valve placement is evenly balanced inside the tester.
6. Remove as much air as possible while filling the chamber with test fluid.
7. Adjust spring compensation as specified in section 6.12 to ensure shaker plate is initially in the middle of the shaker stroke.
8. Connect static head to chamber and fill as specified in section 6.6.
9. Open bypass flow controls.
10. Gradually Increase amplitude to debubbling level. This should be high enough to agitate air out of the system but not high enough to fully close samples.
11. With system in standby, further remove air in system by drawing fluid out of the inflow port using a syringe and allowing air to enter the outflow port capturing the bubbles in the window. Slowly re-introduce fluid into the inflow port allowing air to escape the outflow port.
12. Repeat steps 10 and 11 until all air is removed from the system.

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15.10 Initial Tune

The tuning process can be very different depending on the samples provided. The following is outline of the general procedure for tuning the HiCycle.

1. Check that flow restrictors are still open and that amplitude is at zero.
2. Ensure window screws have a uniform tightness among the various valves, a torque wrench is useful but not necessary.
3. Move pressure transducers to the first chamber – one inflow, one outflow.
4. Ensure HiTest is live and displaying pressures. Set sampling rate according to test plan, the default is 4,000 samples/sec. Set number of cycles in view to allow for appropriate level of cycle detail.
5. Flush transducers with syringe to remove any air bubbles. The pressure tracings should have a relatively smooth trace with minimal high frequency noise.
6. Press standby button to start machine.
7. Gradually increase amplitude until desired opening is achieved using a stroboscope. This will be based on the valve opening during zero-cycle hydrodynamic testing.
8. Turn the flow restrictor until test plan differential requirements are met for the 5% and 95% rule.
9. Recheck valve opening performance, increase amplitude if required.
10. Repeat until requirements and valve opening are acceptable.
11. Repeat steps 7 through 10 for remaining chambers until check revels that all chambers are at desired conditions and no further modifications are required.
12. Capture data and videos using data collection section 15.11.
13. Reset counter after initial tuning and set batch count if applicable. Record the number of cycles accumulated during tuning in the lab notebook.
14. After the completion ensure all data is backed up.

15.11 Data Collection

This section describes the process for testing a single valve. This will have to be repeated for each valve in the durability tester.

1. Click on desired chamber letter corresponding to where the pressure transducers are located
2. Fill in session information in HiTest
    - Trial Title:
    - Trial Number:
    - Valve ID:

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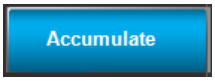
- Fluid Density:
- Fluid Temp:
- Current HiCycle:
- Cycles Counter:
- Operator ID:

3. Record the following in lab note book, if not already done:
   - Test fluid used and relevant parameters if specified in the test plan (viscosity and density)
   - Temperature of test fluid
4. Ensure Amplitude and Restrictor position are recorded.
5. Once system has stabilized with desired parameters click 

6. Once data is collected save in desired test folder.
7. Capture valve opening using a high speed camera. Save videos in desired test folder.
8. Ensure data is being backed up as appropriate.

15.12 Daily Visual Inspection

This is conducted daily unless otherwise specified in the test plan.

1. Remove any accumulated air from the test chamber.
2. Using a stroboscope check the devices for any accumulated damage. Any observations should be recorded. A flash light may also be useful to identify wear. If in doubt use high speed video to characterize the damage.
3. Record the inspection with date, temperature, cycle rate, number of cycles, name, and task completed, or as specified by the test plan.

15.13 Tune Check

Measurements are conducted to ensure valves are meeting the 5% and 95% rule. Checks are to be conducted every 10 million cycles or as specified in the test plan.

1. Move pressure transducers to the first chamber – one inflow, one outflow.
2. Ensure HiTest is live and displaying pressures. Set sampling rate according to test plan, the default is 4,000 samples/sec. Set number of cycles in view to allow for appropriate level of cycle detail.
3. Flush transducers with syringe to remove any air bubbles. The pressure tracings should have a relatively smooth trace with minimal high frequency noise.
4. Follow Data Collection section 15.11 but capture found state and save data files.

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5. If devices are meeting the criteria specified in the test plan and ISO 5840 record these valves. If valves are not meeting the criteria contact the study director before proceeding.
6. If adjustments are required to bring the system back in line with parameters listed in the test plan follow section 0 record the Tune State following section 15.11.
7. Once the system is tuned to the desired conditions, capture high speed video.
8. Once testing is complete have study director sign off.

15.14 Functional Evaluation

At intervals specified in the test plan the valves will be removed from the HiCycle and inspected for wear. At this point additional hydrodynamic assessments may be made.

1. Before removing valves, capture found state and found state high speed videos.
2. Check that temperature on controller is accurate using a calibrated thermometer.
3. Remove valves.
4. Conduct assessment as outlined in 15.8.
5. After the study director has reviewed the data and videos in accordance with the test plan for acceptance criteria including signs of wear or functional impairment, reinstall devices in HiCycle, and conduct tune check as outlined in section 15.13. Ensure valve opening is consistent with the high speed videos captured during the most recent hydrodynamic assessment.

15.15 Data Quality Assurance

Due to the interactive nature of the HiCycle, the following are ideal goals, however every test device is unique and may deviate.

1. Low standard deviations in measurements.
2. Tune states should follow a trend and should not have significant changes in tune state without physical explanations.
3. Pressure signals should be relatively free from noise, which may indicate the transducers were not properly flushed.
4. If similar type and size of devices are being tested, each device should have similar performance characteristics.

15.16 Reporting

Record the following conditions and results. Sections may vary depending on the test plan.

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1. Create a test report.
2. Include a snapshot from high speed video of each valve at both full systole and full diastole.
3. Include any relevant photos taken during valve inspection.

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16. Appendix F - Specifications

HiCycle AR Series	
Capacity	Six test samples
Test Valve Size	Max. diameter 35 mm
Chamber Volume	280 ml
Rate	500-1650 cycles/minute
Operating pressure head	0 - 65 mmHg (8.7kPa)
Electrical Specifications	
Voltage	90-240 VAC / 50-60Hz
Temperature	Ambient to 40 °C ±0.5°C
Dimensions	
Module on Cart	570 x 580 x 1320 mm
Weight (Module)	25 kg
Weight (Controller)	8 kg
Weight (Cart)	18 kg
Environmental	
Operating Temperature	10 to 35ºC
Operating Humidity	15 to 75%
Operating pressure (elevation)	70-106 kPa
Shipping/Storage Temperature	-20 to 50 ºC
Shipping/Storage Humidity	15 to 95%
No restriction on pressure during shipping/storage	

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17. Appendix G - Tool List

The following tools are required to operate a HiCycle AR System. All tools are provided with the system in the Accessories Kit.

HiCycle AR Series Tools
Hex Key 0.05in
Hex Key 5/64in
Hex Key 3/32in
Hex Key 9/64in
Trim Potentiometer Adjustment Tool
Phillips Head Screwdriver
Nut Driver 3/8in

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18. Appendix H - Software License Agreement

The terms and conditions that follow set forth a legal agreement between you (either an individual or an entity), and *ViVitro Labs* (herein referred to as "*ViVitro Labs*") with its principal place of business at 455 Boleskine Road, Victoria, BC Canada V8Z 1E7, relating to the computer software known as *HiTest* and certain other related software Modules, if applicable (herein referred to as "Software").

The term Software includes and these terms and conditions also apply to, any updates or upgrades to the Software that you may receive from time to time under a subscription service or other support arrangement. You may not load or use the Software in any computer or copy it without a license from *ViVitro Labs*. *ViVitro Labs* hereby offers you a non-exclusive license on the terms set out in this Agreement.

You should carefully read these terms and conditions BEFORE opening the case that contains the Software or installing and using the Software. Opening the case containing the Software or installing and using the Software will signify your agreement to be bound by these terms and conditions. If you do not agree to these terms and conditions, promptly return the case containing the Software and the accompanying items (including written materials) for a refund.

This is a license agreement and not an agreement for sale.

18.1 Grant of License

ViVitro Labs grants to you a nonexclusive license to use the Software and the printed and/or electronic user documentation (the "Documentation") accompanying the Software in accordance with this Agreement. If you have paid the license fee for a single user license, this Agreement permits you to use one copy of the Software on any single computer, provided that the Software is in use on only one computer at any time. The Software is "in use" on a computer when it is loaded into the temporary memory (i.e. RAM). If the potential number of users of the Software exceeds the number of licenses you have purchased, then you must have a reasonable mechanism or process in place to assure that the number of computers on which the Software is running concurrently does not exceed the number of licenses purchased.

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You may not take any steps to avoid or defeat the purpose of any such measures. Use of any Software without any required lock device or authorization key is prohibited.

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This Agreement is your proof of license to exercise the rights granted herein and must be retained by you. You may not use any portion of the Software separately from or independently of the Software and other than for your normal business purposes. You may not provide access to or use of the Software to any third party; consequently you may not sell, license, sublicense, transfer, assign, lease or rent (including via an application service provider (ASP) or timeshare arrangement) the Software or the license granted by this Agreement.

You may not modify or make works derivative of the Software and you may not analyze for purposes competitive to *HiTest*, reverse engineer, decompile, disassemble or otherwise attempt to discover the source code of the Software, except in accordance with the following, if applicable, as it contains trade secrets of *ViVitro Labs* and its licensors.

18.2 Term

The license granted herein will continue until it is terminated in accordance with this term. *ViVitro Labs* may terminate the license granted herein immediately upon written notice to you (i) for justified cause, including without limitation breach of any provision of this Agreement, or (ii) if you breach any provision of this Agreement and fail to cure such breach within fifteen (15) days of notice thereof. Upon the termination of the license, you will promptly return to *ViVitro Labs* or destroy all copies of the Software and Documentation covered by the license as instructed by *ViVitro Labs*.

18.3 Responsibility for Selection and Use of Software

You are responsible for the supervision, management and control of the use of the Software, and output of the Software, including, but not limited to: (1) selection of the Software to achieve your intended results; (2) determining the appropriate uses of the Software and the output of the Software in your business; (3) establishing adequate independent procedures for testing the accuracy of the Software and any output; and (4) establishing adequate backup to prevent the loss of data in the event of a Software malfunction.

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18.4 Limited Warranty, Exceptions and Disclaimers

ViVitro Labs warrants that the Software will be free of defects in materials and workmanship and will perform substantially in accordance with the Documentation for a period of ninety (90) days from the date of receipt by you.

ViVitro Labs also warrants that any services it provides from time to time will be performed in a work-person-like manner in accordance with reasonable commercial practice.

ViVitro Labs entire liability and your sole remedy under this warranty shall be to use reasonable efforts to repair or replace the nonconforming media or Software or re-perform the service. If such effort fails *ViVitro Labs* shall (i) refund the price you paid for the Software upon return of the nonconforming Software and a copy of your receipt or the price you paid for the service, as appropriate, or (ii) provide such other remedy as may be required by law. Any replacement Software will be warranted for the remainder of the original warranty period or thirty (30) days from the date of receipt by you, whichever is longer.

b. Exceptions.

ViVitro Labs limited warranty is void if breach of the warranty has resulted from (i) accident, corruption, misuse or neglect of the Software; (ii) acts or omissions by someone other than *ViVitro Labs*; (iii) combination of the Software with products, material or software not provided by *ViVitro Labs* or not intended for combination with the Software; or (iv) failure by you to incorporate and use all updates to the Software available from *ViVitro Labs*. *ViVitro Labs* does not warrant that the Software or service will meet your requirements or that the operation of the Software will be uninterrupted or error free.

c. Limitations on Warranties.

The express warranty set forth in 18.4 is the only warranty given by *ViVitro Labs* with respect to the Software and Documentation furnished hereunder and any service supplied from time to time; *ViVitro Labs* and its licensors, to the maximum extent permitted by applicable law, make no other warranties, express, implied or arising by custom or trade usage, and specifically disclaim the warranties of merchantability and fitness for a particular purpose.

In no event may you bring any claim, action or proceeding arising out of the warranty set forth in 18.4 more than one year after the date on which the breach of warranty occurred.

d. Limitations on Liability.

Except as required under local law, the liability of *ViVitro Labs* and its licensors, whether in contract, tort (including negligence) or otherwise, arising out of or in connection with the Software or Documentation furnished hereunder and any service supplied from time to time shall not exceed the license fee you paid for the Software or any fee you paid for the service. In no event shall *ViVitro Labs* or its licensors be liable for special, indirect, incidental, punitive or consequential damages (including without limitation damages resulting from loss of use, loss of data, loss of profits, loss of goodwill or loss of business)

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arising out of or in connection with the use of or inability to use the Software or Documentation furnished hereunder and any service supplied from time to time, even if *ViVitro Labs* or its licensors have been advised of the possibility of such damages. Notwithstanding the foregoing, in no event shall *ViVitro Labs*' liability be limited in the case of death or personal injury arising as a result of the negligence or willful misconduct of *ViVitro Labs*.

18.5 European Software Directive

If the provisions of the Council of European Communities Directive of May 14, 1991 on the Legal Protection of Computer Programs as implemented in applicable national legislation (the "Software Directive") apply to your use of the Software, and you wish to obtain the information necessary to achieve interoperability of an independently created computer program with the Software as permitted under the Software Directive ("Interoperability Information"), you must notify *ViVitro Labs* in writing, specifying the nature of the Interoperability Information you need and the purpose for which it will be used.

If *ViVitro Labs* reasonably determines that you are entitled to such Interoperability Information under the Software Directive, *ViVitro Labs* Inc shall, at its option, either (i) provide such Interoperability Information to you, or (ii) allow you to reverse engineer the Software, within the limits and for the purposes prescribed by the Software Directive, solely to the extent indispensable to obtain such Interoperability Information. If *ViVitro Labs* Inc elects clause (i), you will provide any information and assistance reasonably requested by *ViVitro Labs* Inc to enable *ViVitro Labs* Inc to perform clause (i), and *ViVitro Labs* Inc may charge you a reasonable fee for making available the requested Interoperability Information, unless such a fee is prohibited under the Software Directive.

18.6 General Provisions

You acknowledge that the Software and the Documentation may be subject to the export control laws of the United States or the United Kingdom and agree not to export or re-export the Software or the Documentation (i.e., move the Software from the country in which you first licensed it) without the appropriate United States or foreign government licenses and the written approval of *ViVitro Labs* Inc and its licensors. The United Nations Convention on Contracts for the International Sale of Goods (1980) is here by excluded in its entirety from application to this License Agreement. The English language version of this Agreement shall be the authorized text for all purposes, despite translations or interpretations of this Agreement into other languages. If for any reason a court of competent jurisdiction finds any provision of this Agreement, or a portion thereof, to be unenforceable, that provision shall be enforced to the maximum extent permissible and the remainder of this Agreement shall remain in full force and effect.

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18.7 Canadian Sales

If you purchased this product in Canada, you agree to the following:

The parties hereto confirm that it is their wish that this Agreement, as well as other documents relating hereto, including Notices, have been and shall be written in the English language only.

Les parties aux présentes confirment leur volunté que cette convention de même que tous les documents y compris tout avis qui s'y rattache, soient rédigés en langue anglaise.

You further agree that this Agreement is the complete and exclusive statement of your agreement with *ViVitro Labs* Inc relating to the Software and subscription service and supersedes any other agreement, oral or written, or any other communications between you and *ViVitro Labs* Inc relating to the Software and subscription service; provided, however, that this Agreement shall not supersede the terms of any signed agreement between you and *ViVitro Labs* Inc relating to the Software and subscription service.

ViVitro Labs warrants all parts of its own manufacture to be free from defects in material and workmanship under normal use and service in a suitable environment for the period of one year after delivery.

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